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Low-dose Herbicide Effects on Tree Establishment and Soil Nitrogen Biogeochemistry within Pine Savannas

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Longleaf pine (*Pinus palustris* P. Mill) survival and growth, net nitrogen mineralization, and soil microbial biomass, were evaluated after four growing seasons in a Florida wet flatwoods site following chemical vegetation control during the first year or second year after planting, or during both years. The four herbicide treatments included sulfometuron methyl at 0.26 ai kg ha^{-1}, hexazinone (0.56 ai kg ha^{-1}), sulfometuron (0.26 ai kg ha^{-1}) plus hexazinone (0.56 ai kg ha^{-1}) mix, and imazapyr at 0.21 ai kg ha^{-1}. Imazapyr was the only treatment to significantly improve growth over the control in a single application. Consecutive annual applications of imazapyr and hexazinone on seedlings also improved growth rates compared to the control. Sulfometuron methyl-treated pine trees had lower survival rates and were smaller than pines growing in the control plots after a single application. The survival and growth rates of imazapyr-treated seedlings were improved when the chemical was applied during the second growing season after planting, instead of the first year. Imazapyr and hexazinone applications increased net nitrogen mineralization rates, but imazapyr was the only treatment to increase ammonification; compared to the control. Microbial and fungal biomass carbon showed no differences between treatments. The results did show that microbial biomass significantly increased with two consecutive years of herbicide applications over a single application. Imazapyr applied during the second growing season proved to be the best treatment for improving pine growth, controlling competitive vegetation, minimizing pine mortality, and to remain effective when soils are saturated.

Core Ideas

- **Imazapyr-treated pines were larger than pines applied with other herbicides.**
- **Imazapyr-treated pines had better survival when the treatment is applied during the second season.**
- **Imazapyr soils produced higher ammonification rates compared to the control.**
- **Sulfometuron methyl-treated pines were smaller than control plot trees.**
- **Sulfometuron methyl-treated soils had lower ammonification rates and microbial biomass.**

The "grass" stage of longleaf pine (*Pinus palustris* P. Mill) development and the Gulf coastal environment in which the pines grow are two factors which distinguish much of Florida's longleaf pine forests from pine ecosystems of other regions of the United States. Longleaf pine regeneration is dependent on a "grass" stage when the pine seedlings are able to survive light surface fires and fierce vegetative competition (Keeley, 2012; Pessin, 1934). Longleaf pine seedlings have been reported to stay in the grass stage up to 15 yr, depending on the environmental conditions. This "dormant" state can make the growth rates of longleaf pines highly unpredictable (Haywood, 2000; Keeley, 2012). Second, Gulf coastal wet flatwoods are unique habitats because they are located on low, rain-fed coastal terraces where weather patterns maintain high soil moisture conditions for extended periods during the growing season (McCaskill and Jose, 2012; Messina and Conner, 1998). These two factors along with the application of herbicides make it imperative to research the soil biogeochemical properties of these ecosystems during restoration (Harris, 2003; Heneghan et al., 2008; Johnston and Crossley, 2002; McCaskill et al., 2018).

The Point Washington restoration study was initiated in 2001 to convert an existing 26-yr-old slash pine plantation located on Florida's Gulf coast, back to a longleaf pine flatwoods ecosystem (Fig. 1). The effects of a single low-dose application of four chemical treatments on longleaf pine growth and survival, and un-

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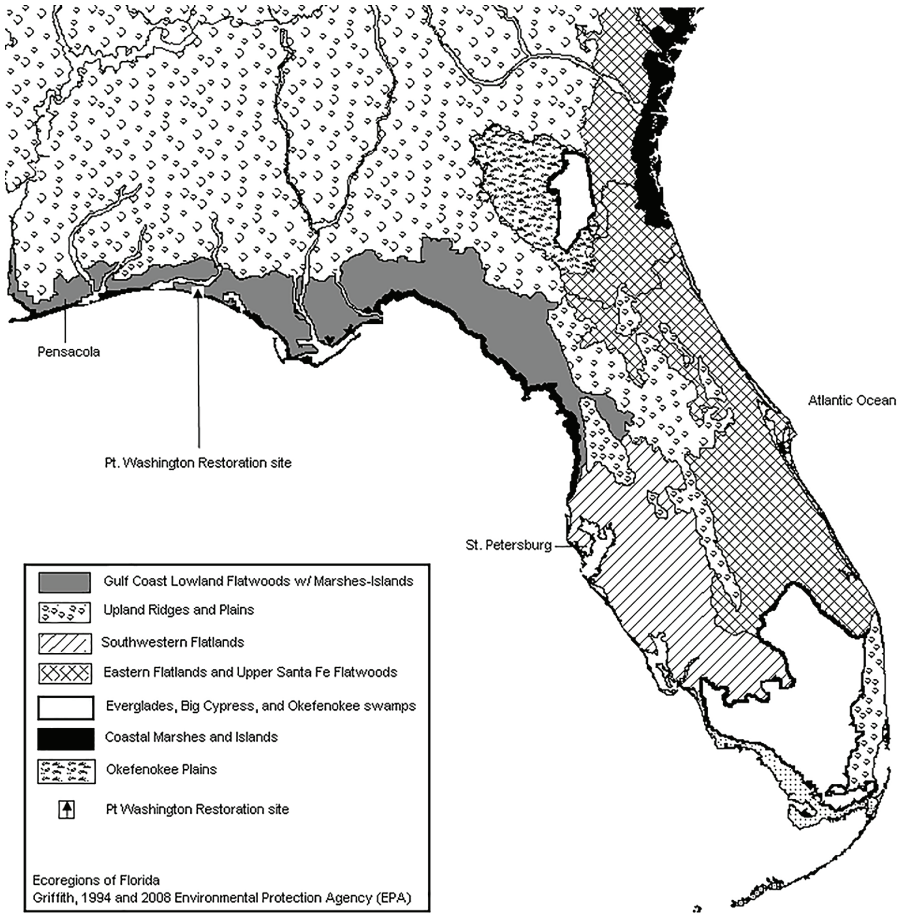

Fig. 1. Location of the Point Washington restoration site within the Gulf Coast Flatwoods zone of Florida, USA (Griffith et al. 1994, 2008).

derstory plant species richness were evaluated after one growing season (Jose et al., 2008; Ranasinghe, 2003), as well as four growing seasons followed by a second burn (Freeman and Jose, 2009).

In 2003, the four treatments were reapplied to some of the rows during the second growing season after planting to establish consecutive year treatments (McCaskill, 2008). Only imazapyr and hexazinone were applied to some of the control plots for establishing second year-only treatments. The project was also expanded to include the addition of soil biochemical assays for determining the impacts of chemical treatments on nitrogen (N) cycling (McCaskill et al., 2018). The main objectives of the expanded study were to determine the effects of applying the original herbicide treatments in the second year after planting compared to first-year treatments, consecutive herbicide treatments (first and second years), and whether a March or April application changes the herbicide effects on longleaf pine development during the second growing season. Questions asked were: (1) How did changing the timing and the number of applications effect the impacts of the four original chemical treatments on pine growth and survival? (2) Would the soil biogeochemical measurements provide a means to detect differences between treatment effects on soil N within this wet longleaf pine habitat? The answers to these questions will provide a greater knowledge concerning the assessment of vegetative control efforts applied in coastal wet pine flatwoods.

MATERIALS AND METHODS

Point Washington Restoration Site

The longleaf pine restoration project was located on the Point Washington State Forest (30°20′ N, 86°4′ W) in southern Walton County, Florida, USA (Fig. 1; Freeman and Jose, 2009; Jose et al., 2008; McCaskill, 2008). This coastal wet pine flatwoods site was approximately a 4-ha, 26-yr-old slash pine plantation having an average basal area of 7.85 m^2 ha^{-1} and an average diameter-at-breast height (dbh) of 19.1 cm, as measured in 2001. It also contained residual longleaf pine saplings and pole trees as part of the stand's original stocking. The adjacent area is comprised of approximately 20 ha of mixed slash and longleaf pine surrounding a large cypress dome and contained within the greater 6800-ha Point Washington State Forest.

The understory plant community was dominated by *Andropogon virginicus*, a smaller component of *Aristida stricta*, and a group of shrub species highlighted by *Ilex glabra, Serenoa repens*, *Quercus pumila*, and *Gaylussacia frondosa*. Wetter areas had a greater presence of *Lachnanthes caroliniana, Cliftonia monophylla*, *Nyssa sylvatica* var. *biflora*, *Cyperus*, *Scleria*, *Xyris*, and *Lachnocaulon* (Freeman and Jose, 2009; McCaskill, 2008; McCaskill and Jose, 2012; Peet and Allard, 1993). The annual precipitation averages 1500 mm with most of it occurring during the late summer. Soils belong to the Leon series and are classified as sandy, siliceous, thermic, aeric Alaquods. They are described as highly weathered, poorly drained, acidic, infertile substrates (McCaskill and Jose, 2012; Overing and Watts, 1989). This pine flatwoods is found very close to the coast (within 3 km) where its soils were formed on sandy quaternary parent material derived from marine deposits (Stout and Marion, 1993).

Site Treatments

An overstory of slash pine (4 ha) was clear-cut during August 2001, roller chopped once, and prescribed burned in October 2001. No existing bedding or any other hydrological-modifying practice was applied. Six experimental blocks were placed within the 4-ha burned area. Each block contained four plots representing each of the herbicide treatments, along with one control plot. In December 2001, 100 (1-yr-old) containerized longleaf pine seedlings were hand-planted at 3.1 m by 1.8 m spacing into ten rows per treatment plot. A total of 3000 seedlings were planted within the six experimental blocks. In March 2002, four herbicide treatments were applied in a 1.2-m band over the top of the

pine seedlings using a knapsack sprayer. These treatments were evaluated under a complete randomized block design (Freeman and Jose, 2009; Jose et al., 2008).

In 2003, the four original herbicide treatments were re-applied over the top of pine seedlings using the same dosages for the purpose of evaluating additional herbicide treatments applied during the second growing season after planting, the effects of consecutive herbicide treatments (first and second year), and whether a March or April application during the second growing season changes the rate of longleaf pine development. Sulfometuron, c(methyl 2-[[[[(4, 6-dimethyl-pyrimidinyl) amino] carbonyl] amino]sulfonyl]benzoate) methyl (manuf. name Oust XP), was applied at 0.26 ai kg ha^{-1}, while hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione) (manuf. name Velpar L) was applied at 0.56 ai kg ha^{-1}, and a mixture of sulfometuron methyl (0.26 ai kg ha^{-1}) and hexazinone (0.56 ai kg ha^{-1}) was applied as a third treatment. Imazapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid) (manuf. name Arsenal) was applied at a lower 0.21 ai kg ha^{-1}, representing the fourth treatment.

The five original plots per experimental block became split-plots (each plot with three nested treatments). The first three rows (30 trees) of each treatment plot were repeated in March and the second three rows resprayed in April of 2003, representing two consecutive years. The final four rows were left as the original single-year treatment. The original control plots were split leaving the first four rows as the untreated control. The second three rows of 30 trees in the control plots were sprayed in March with hexazinone in Blocks 1, 2, and 5; and with imazapyr in Blocks 3, 4, 6, representing second year-only treatments. The final three rows of trees within the control plots were sprayed with the same treatments, but applied in April, representing a late spring application of the second year-only treatments (McCaskill, 2008). The impact of applying the second-year chemical applications to the original experimental design resulted in a modification to represent a split-plot randomized unbalanced incomplete-block design, requiring a (Studnicki et al., 2015) mixed model analysis for both fixed and random effects (Chen and Deo, 2010).

Pine Survival and Growth

Pine growth and survival were monitored at the end of the growing season every year, through 2006 (McCaskill, 2008). Seedling and sapling heights (cm) were measured from the base at soil surface to the top of the terminal bud using a meter stick. Root collar diameter was measured (mm) using a digital caliper. Stem volume index was calculated (cm^3) with the measured root collar diameter and stem height data (Elliot and Vose, 1995; Momen et al., 2002).

Soil Sampling and Preparation

Soil samples (90) were taken from the top 10 cm monthly within experimental Blocks 1, 4, and 6 during 2002 and 2003; the time period covering the two chemical applications. Samples were also collected from all six experimental blocks during spring and summer of 2005 and 2006 for microbial and fungal biomass analysis (McCaskill et al., 2018). All of the samples were immediately stored at 4°C until analysis. Subsamples (20 g) were analyzed for soil pH by prepared slurries using a soil/water ratio of 1:2 (McLean, 1982), percent organic matter content by the Walkley–Black method (Walkley, 1947), and a sieved and dried (105°C) subsample was used to determine gravimetric moisture content.

In Situ Net Nitrogen Mineralization

Net N mineralization rates were determined by in situ incubation described by Eno (1960) on the same 90 soil samples over the 14-mo treatment period. Mineral N was extracted from 20 g of soil according to Keeney and Nelson (1982). A total of 180 samples were analyzed for ammonium (NH_4^+) and nitrate (NO_3^-) using a continuous auto-flow analyzer. Net N mineralization was calculated as the difference between incubated- and initial-N mineralization, corrected for soil moisture (Keeney and Nelson, 1982; McCaskill et al., 2018; McCaskill and Jose, 2012).

Microbial and Fungal Biomass

Soil microbial biomass carbon was determined by chloroform fumigation-extraction of the soil samples collected during 2005 to 2006, according to Vance et al. (1987). A lower 0.05 M K_2SO_4 extractant was used to remove carbon from the control (non-fumigated) and fumigated soil samples taken from the low pH soils (Haney et al., 2001). Extraction required the samples to be shaken for 1 h and centrifuged (6000 rpm) for 15 min before filtering the resultant supernatant through Whatcom no. 42 filter paper. Total organic carbon (TOC) was determined on a Shimadzu TOC-VCSH analyzer according to Vance and Entry (2000). Microbial biomass carbon was calculated as: mg C_{MB} kg $soil^{-1}$ = [($TOC_{fumigated}$ – $TOC_{control}$)/0.51]/(soil dry mass) (Joergensen, 1996; McCaskill et al., 2018).

Soil fungal biomass carbon was determined by the extraction of ergosterol from the same soil samples (Gong et al., 2001). Ergosterol was extracted with methanol (0°C) from each sample by shaking for 1 h at 360 rev min^{-1} and centrifuged at 11,000 rpm for 20 min. The supernatant (1.5 mL) was measured by High Performance Liquid Chromatography (HPLC) at 282 nm (Beckman Coulter System Gold HPLC) and expressed in micrograms (μg) ergosterol per gram of soil. An averaged conversion factor for 3.65 μg of ergosterol per milligram of soil translates to fungal biomass carbon (mg C_{FB} g $soil^{-1}$) when multiplied by 220 (McCaskill et al., 2018; Montgomery et al., 2000). Soil microbial biomass assays were performed on a total of 48 soil samples incorporating six replications (n = 8 per experimental block).

Data Analysis

The resulting split-plot randomized unbalanced incomplete-block design required an analysis utilizing PROC MIXED procedure in SAS 2014 (SAS Institute, 2014) incorporating the Residual Maximum Likelihood (REML) function to process non-normal data that was unbalanced, and to detect any inter-

Table 1. Measurements after 4 yr of growth for average root collar diameters, tree heights, and calculated stem volume taken from longleaf pine trees within the control plots by experimental block at the Port Washington State Forest in Florida, USA.

Block	Root collar diameter	Height	Stem volume index
	mm		cm^3
1	47.9	876.5	2724.8
2	22.9	76.7	52.8
3	23.9	78.1	78.1
4	31.0	201.4	377.5
5	31.6	135.2	177.6
6	21.2	67.9	36.1

actions between the experimental blocks and treatments (Chen and Deo, 2010; Momen et al., 2002; Studnicki et al., 2015).

The mixed-model fixed effects were root collar diameter, tree height, stem volume index, and the 16 combination treatments, while the random effects were the six blocks, with five plots per block, and the number of tree rows per treatment; within the framework of the incomplete split-plot block design (Studnicki et al., 2015). Significant treatment effects were determined using differences between Least Squared Means (LSM; Chen and Deo, 2010). Hypothesis testing for differences between the treatments and the control was accomplished by using Dunnett's Hsu *t* test for multiple means comparison with an α of 0.05 and a two-tailed confidence interval of 95% (Ott and Longnecker, 2001).

Soil N mineralization fluxes were also analyzed using a mixed-model (proc mixed) incorporating Residual Maximum Likelihood (REML) analysis to estimate treatment effects on N fluxes and to detect any interactions between the experimental blocks and the four herbicide treatments (Ross et al., 1999; Butt et al., 2005). Significant treatment effects on the soil biogeochemical indicators were determined using differences between Least Squared Means (LSM; Chen and Deo, 2010). Hypothesis testing for differences between the treatments and the control was accomplished by using Dunnett's Hsu *t* test for multiple means comparison with an α of 0.05 and a two-tailed confidence interval of 95% (Ott and Longnecker, 2001).

RESULTS AND DISCUSSION

Pine Survival and Growth

Two of the five plots within Block 1 produced a group of longleaf pine saplings substantially taller than any of the pine plantings from the other five experimental blocks, regardless of treatment (Table 1). Therefore, Block 1 was eliminated from the analysis to unmask the differences between treatment effects. The March versus April applications during the second growing season failed to produce any significant differences within each of the treatments, and therefore were combined either as 2-yr or second-year-only treatments, strengthening the statistical inference produced by having larger sample sizes.

Imazapyr-treated pine trees had significantly lower survival rates compared to the control or the hexazinone-treated trees (Fig. 2). But the results also showed the survival rates for the imazapyr-treated trees were comparable to the control trees when the chemical was applied during the second growing season, instead of the first season. Sulfometuron methyl applied during the first growing season and sulfometuron methyl plus hexazinone treatments produced lower survival rates than the control trees.

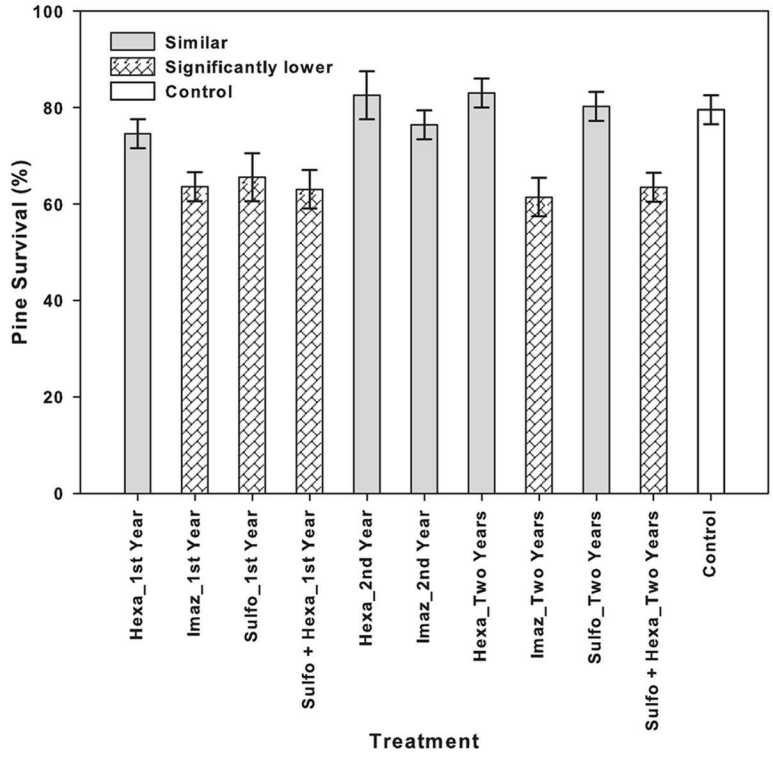

Fig. 2. Pine survival (%) for the control, hexazinone ("Hexa"), imazapyr ("Imaz"), sulfometuron methyl ("Sulfo"), and Sulfo + Hexa mixture. Results are from three growing seasons after second-year treatments. Error bars indicate significantly different values ($p < 0.05$).

The imazapyr-treated trees had significantly larger mean root collar diameters, pine heights, and stem volume index values compared to the control trees (Fig. 3).

Specifically, imazapyr applied during the second growing season produced the largest heights and stem volume index values. Imazapyr applied in consecutive years was comparable to second year-only treatments (Fig. 3; Table 2). Hexazinone applied in consecutive growing seasons also produced higher root collar diameters and stem volume index values compared to the control. The REML mixed-model analysis produced results indicating only imazapyr, and to a lesser degree, hexazinone treatments produced trees significantly larger than the control trees. Sulfometuron methyl applied during the first growing season was the only treatment to produce lower mean survival rates, root collar diameters, pine heights, and stem volume index values when compared to the control.

Soil Biogeochemical Properties

The highest mean monthly N mineralization rates were observed in the imazapyr-treated plots (Fig.

4). Imazapyr and hexazinone-treated plots had higher N mineralization rates when compared to the control through the REML mixed-model analysis (Table 3). Imazapyr was the only treatment to produce significantly higher ammonification estimates compared to the control. The sulfometuron methyl-treated plots produced significantly lower ammonification rates than the control or imazapyr plots (Fig. 4). The sulfometuron methyl results were not significant through the REML mixed-model analysis. The hexazinone-treated plots produced highly variable data for the N mineralization and ammonification estimates. The sulfometuron methyl plus hexazinone mix did produce significantly higher nitrification rates than the control. Sulfometuron methyl treatments also produced the lowest soil microbial biomass concentrations, that were only significantly different from the hexazinone and sulfometuron methyl-hexazinone treatments (Fig. 5). Except for the sulfometuron methyl treatments, microbial or fungal biomass carbon concentrations were not significantly different among the other herbicide treatments or the control. The results did show that microbial biomass significantly increased with two consecutive years of herbicide applications over a single application (Fig. 5).

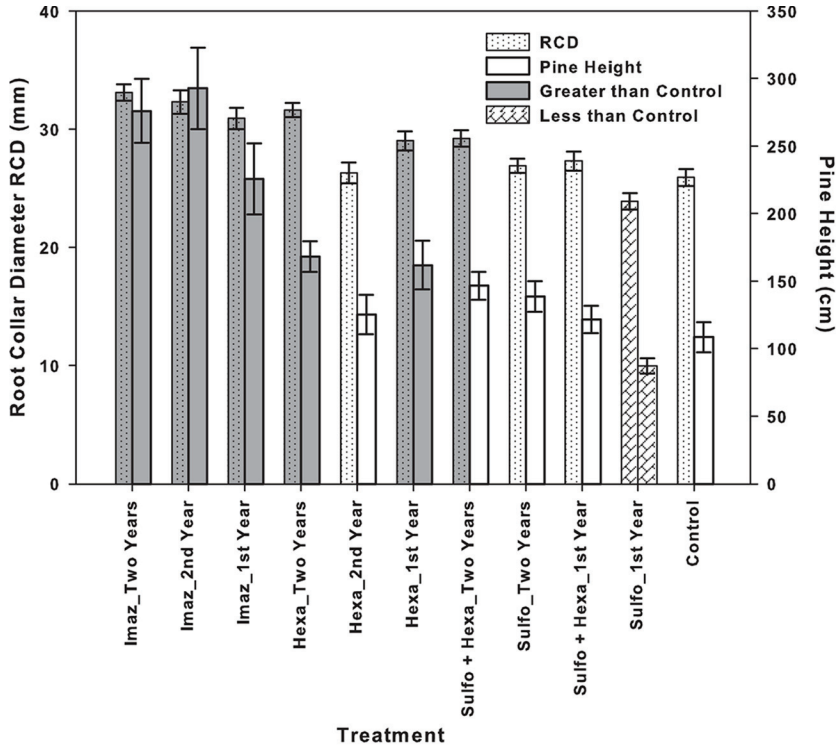

Fig. 3. Pine root collar diameters, tree height, and stem volume index for the control, hexazinone ("Hexa"), imazapyr ("Imaz"), sulfometuron methyl ("Sulfo"), and Sulfo + Hexa mixture. Results are from three growing seasons after second-year treatments. Error bars indicate significantly different values ($p < 0.05$).

Most herbicides are readily broken down by soil microbes causing an increase in microbial numbers and activity (Haney et al., 2002). The application of herbicides during the first year of longleaf pine establishment has also proven to increase growth in previous studies (Ramsey et al., 2003). However, higher pine mortality associated with some of the chemicals such as imazapyr has been a concern. Earlier results from this study based on the original four herbicide treatments applied during the first growing season and evaluated after 4 yr of pine growth showed imazapyr, followed by hexazinone treatments, had produced significantly higher numbers of pine seedlings in the out-of-the-grass stage (>120 cm height) when compared to the other treatments (Freeman and Jose, 2009; Jose et al., 2008). These earlier studies also found imazapyr treatments resulted in the best control of the overall cover (%) and stem counts of the major shrub species, while producing the highest level of herbaceous richness (Freeman and Jose, 2009; Jose et al., 2008). These gains were offset by imazapyr producing one of the lowest longleaf pine seedling survival rates.

The results verified a recommendation (BASF Corp., Research Triangle Park, NC) that survival and growth of imazapyr-treated pine seedlings were significantly improved if the chemical was applied during the second growing season, instead of the first season (Table 2; Fig. 2; Fig. 3; BASF Corporation, 2015).

Table 2. Differences in least-square means (±SE) of stem volume index between herbicide treatments and the control for longleaf pine at the end of four growing seasons on the Port Washington State Forest in Florida, USA.

Treatment†	Stem volume index	df	*p*‡
Sulfo first year vs. Control	−87.4 (42.8)	122	0.0433
Sulfo two years vs. Control	86.5 (50.0)	250	ns§
Hexa first year vs. Control	79.4 (50.7)	148	ns
Hexa two years vs. Control	91.9 (38.7)	250	0.0187
Sulfo + Hexa first year vs. Control	−32.5 (44.7)	124	ns
Suflo + Hexa two years vs. Control	71.6 (43.7)	186	ns
Imaz first year vs. Control	225.4 (80.5)	127	0.0059
Imaz two years vs. Control	309.7 (68.9)	184	<0.0001
Hexa second year vs. Control	15.4 (56.5)	99	ns
Imaz second year vs. Control	375.8 (15.7)	125	<0.0001

† Hexa, hexazinone; Imaz, imazapyr; Sulfo, sulfometuron methyl.

‡ Least-square mean comparison of post-planting treatments with the control ($P < 0.05$) using Dunnett's Hsu.

§ ns, no significance.

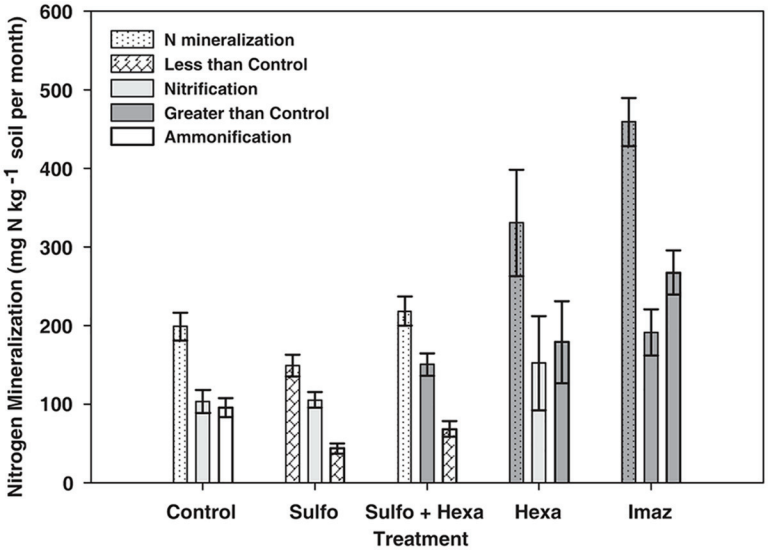

Fig. 4. Net N mineralization (mg NH_4^+ kg soil^{-1} mo^{-1} + mg NO_3^- kg soil^{-1} mo^{-1}) for the control, sulfometuron methyl ("Sulfo"), hexazinone ("Hexa"), Sulfo + Hexa mixture, and imazapyr ("Imaz"). Results are from monthly soil samples taken from January 2001 through April 2003. Error bars indicate significantly different values ($p < 0.05$).

If imazapyr, a leucine and isoleucine protein synthesis inhibitor, was the only treatment to produce significantly higher longleaf pine growth as well as ammonification rates when compared to the control, then some factor must have partially interfered with the effects of the other herbicide treatments on pine growth and N mineralization.

The concerns for hexazinone, a photosystem II quinone inhibitor, are mobility in moist soils and persistence in water. It was also found to inhibit ammonification and stimulate denitrification, dominant transformations during flooding events (Bayer Environmental Science, 2018b; McCaskill et al., 2018; Rolando et al., 2013; Vienneau et al., 2004). The chemical has also been found to inhibit mycelia formation in mycorrhizal fungi, a major transport system for ammonium (Estok et al., 1989; Karpouzas et al., 2014; Litten et al., 1985). Sulfometuron methyl, an acetolactate synthase inhibitor, has been found to quickly move off-site when applied to sites in contact with water (Bayer Environmental Science, 2018a; Michael et al., 2006). The chemical has also been found to be toxic in low concentrations to many strains of *Pseudomonas*, a genus of heterotrophic bacteria prominently involved with ammonification, and to ammonium-oxidizing (AOB) bacteria involved with nitrification (Boldt and Jacobsen, 1998; Burnet and Hodgson, 1991; Gedi and Moon-Young, 2012; Marileo et al., 2016; Whitcomb, 1999). This finding might explain the low rates of ammonification and microbial biomass carbon attributed to the application of sulfometuron methyl. How these factors relate to sulfometuron methyl treatments producing poor pine growth and lower pine survival rates requires further research. The chemical properties of hexazinone and sulfometuron methyl when in contact with water, limit their effects on vegetative control within this coastal wet pine flatwoods site where the sandy soils (Leon series) have a moderate soil leaching rating and a high soil runoff rating for pesticide selection (Langeland et al., 2006; McCaskill and Jose, 2012; Obreza and Hurt, 2006).

The two main reasons why imazapyr was more effective than the other herbicide treatments in reducing vegetation competition without significantly impacting species richness within this wet pine site are due to its properties. First, imazapyr is a broader-spectrum herbicide and more effective at controlling perennial woody species. This property is critical in mimicking fire effects. Second, imazapyr is more persistent in wet sandy soils where hexazinone or sulfometuron methyl are transported away with rain events (BASF Corporation, 2015; Gianelli et al., 2014). This is also a critical factor in wet longleaf pine sites where the water table is constantly near the surface and the effects of herbicide treatments can be reduced by flooding. It should be noted that imazapyr is the only chemical among this group of herbicides to be currently certified by the EPA for use in aquatic systems (Habitat [SePRO Corporation, 2016]).

Measurement of microbial and fungal biomass carbon was ineffective at detecting differences between the herbicide treatments. The results did show that microbial biomass increased with 2 yr of applications, indicating microbial populations were stimulated by chemical biodegradation. A previous study also failed to detect any herbicide effects on fungal communities (Busse et al., 2004). But, a primary reason for the failure of microbial biomass and fungal biomass carbon concentrations to detect statistically significant differences between the treatments and the control might be attributed to the 2-yr time period between the second-year treatments and the microbial tests. This time period may have provided a recovery period from the chemical impacts of herbicides on microbial numbers (Li et al., 2003). Had earlier soil sampling included soil microbial analysis utilizing repeated measurements, our ability to detect differences between treatments would have been strengthened, but at a much greater expense. Finally,

Table 3. Differences in least-square means (±SE) for N mineralization and ammonification rates for post-planting chemical treatments versus the control on a longleaf pine flatwoods ecosystem in Florida, USA.

Treatment†	Nitrogen mineralization	df	*p*	Ammonification	df	*p*‡
	mg N kg soil^{-1}			mg NH_4^+ kg soil^{-1}		
Sulfo vs. Control	44.1 (26.9)	270	ns§	28.6 (28.7)	294	ns
Hexa vs. Control	76.6 (25.7)	270	0.0031	33.6 (26.8)	294	ns
Sulfo + Hexa vs. Control	20.8 (26.3)	270	ns	−3.3 (25.6)	294	ns
Imazapyr vs. Control	53.1 (24.8)	280	0.0333	68.2 (27.0)	294	0.0122

† Hexa, hexazinone; Imaz, imazapyr; Sulfo, sulfometuron methyl.

‡ Least-square mean comparison of post-planting treatments with the control ($P < 0.05$) using Dunnett's Hsu.

§ ns, no significance.

what caused a crop of "super trees" to appear in two of the five plots from Block 1 remains a mystery. There may have been the introduction (a spill) of some chemical or nutrients prior to the establishment of the experimental blocks, or maybe it was simply a superior strain of pine seedlings planted in that particular block? In any case, Block 1 had to be eliminated from the analysis to reduce any statistical "noise" that would prevent the ability to evaluate differences between herbicides effects on pine survival and growth.

CONCLUSIONS

These results indicated growth benefits can be achieved without compromising survival if imazapyr applications are delayed until the second growing season after planting. Sulfometuron methyl treatments have negative effects on pine growth and survival when applied on this wet site, irrespective of the year or time. Hexazinone is only marginally effective with consecutive applications and produces highly variable results brought on by flooding events.

The application of imazapyr, and to a less degree, hexazinone; stimulated increases in N mineralization rates, but imazapyr was the only treatment to have significantly higher levels of ammonification compared to the control. Sulfometuron methyl treatments produced significantly lower ammonification rates compared to imazapyr or the control. Microbial and fungal biomass measurements were ineffective in delineating differences among the treatments. Microbial biomass increased with two herbicide applications over a single application.

Overall, imazapyr applied during the second-year growing season proved to be the best vegetative control treatment based on its ability to improve pine growth while limiting mortality, and to remain effective during flooding events.

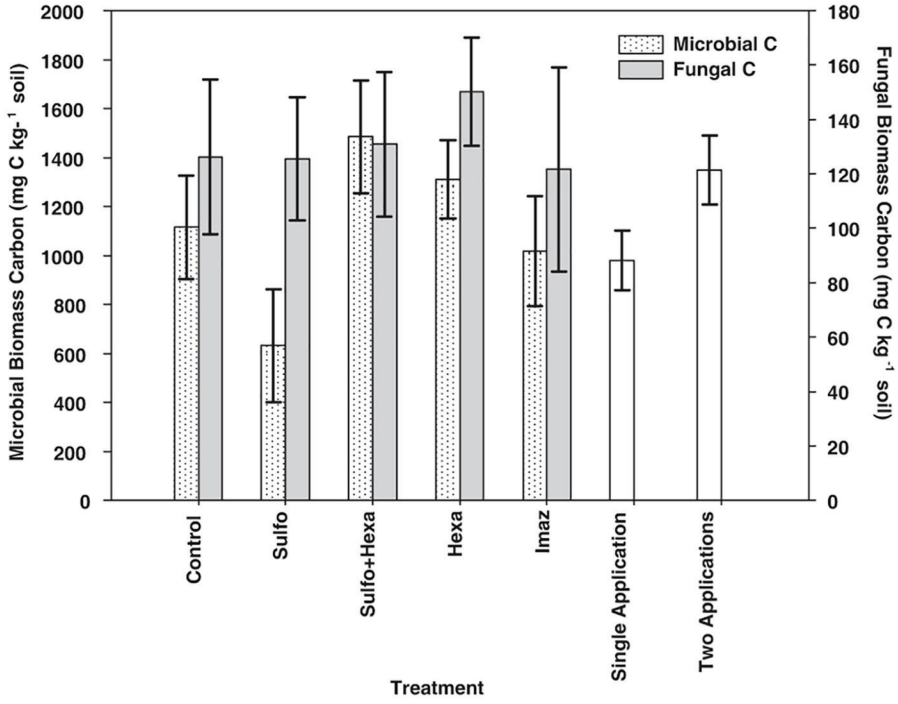

Fig. 5. Microbial and fungal biomass carbon (mg C kg soil^{-1}) for the control, sulfometuron methyl ("Sulfo"), hexazinone ("Hexa"), Sulfo + Hexa mixture, and imazapyr ("Imaz"). Results are from soil samples taken in April and August 2005 and 2006. Error bars indicates significantly different values ($p < 0.05$).

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