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MOISTURE RETENTION, AND PHOTOSYNTHETIC INTRINSIC WATER USE
EFFICIENCY IN THE FIELD**

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Pyrogenic Carbon Increases Pitch Pine Seedling Growth, Soil Moisture Retention, and Photosynthetic Intrinsic Water Use Efficiency in the Field

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Climate change and land management are altering forest fire frequency and intensity worldwide. In some Northeast U.S. forests, pitch pine (*Pinus rigida* Miller) is not suffering from presence but rather a lack of wildfire events. In their absence, prescribed fire is being used to diminish fuel loads, open canopies and reduce competition. Pyrogenic carbon (PyC) produced by the fires may also improve soil moisture retention and plant physiological processes. Where the application of prescribed fire is not feasible due to nearby human populations, we reason prescribed fire PyC could be replaced by anthropogenic PyC product to provide similar soil benefits. We tested this hypothesis with pitch pine seedlings at a site absent overstory planted in submerged tree pots with control and PyC-imbued soils. Investigators found anthropogenic and forest PyC fostered similar growth, soil moisture retention and photosynthetic intrinsic water use efficiency, both significantly higher than unamended soils. We conclude anthropogenic subsurface PyC soil amendment provides a conservation management tool for enhancing benefits in ecosystems where prescribed fire is not a viable option in northerly forests in the U.S.

Keywords: PyC, prescribed fire, charcoal, forest ecology, soil moisture, water use efficiency, conservation

INTRODUCTION

Forest fire frequency and intensity are being altered by climate change, notably in northern latitudes (Liu et al., 2019). Pitch pine (*Pinus rigida* Miller) species, adapted to wildfire perturbation (Patterson and Backman, 1988; Parshall et al., 2003), face increased hardship from an absence of stand-replacing fire (Foster and Motzkin, 2003; Nowacki and Abrams, 2008) which no doubt contributes to pressure on pitch pine vigor (Patterson et al., 1983). Warmer temperatures and changes in precipitation patterns might possibly tip the balance toward greater competitiveness except for the fact that pitch pine competitors are not as dependent on open canopies (Fischer et al., 2013), are more resistant (Lesk et al., 2017) to southern pine beetle (*Dendroctonus frontalis*) and appear to be more adaptive and resilient to climate change (Swanston et al., 2018).

In place of forest fire, prescribed fire (Little, 1953; Neill et al., 2007) is employed to open overstories, reduce fuel availability and eliminate competition from other species. Prescribed fire pyrogenic carbon (PyC), essentially charcoal, disperses into O_a-A_b horizons (Neill et al., 2007) leaving little doubt of its indelible mark on juvenile trees. Much is known about the effects of

forest fire and prescribed fire PyC (Doerr et al., 2018). Notably, prescribed fire (Foereid et al., 2015; Michelotti and Meisel, 2015) produces highly reactive and highly sorbent material that enrich forest soils (Thomas and Gale, 2015; Mitchell et al., 2016). However, less understood are anthropogenic PyC impacts on forest soil properties and tree growth (Ge et al., 2019), especially juvenile trees. Some studies focus on PyC and tree germination processes (McElligott, 2011; Ledig et al., 2015, but few if any describe post-germination impacts and none on seedling growth of a particular species pitch pine.

PyC efficacy is measured in terms of soil moisture sorption and retention (Licht et al., 2017), which influence growth potential. Forest PyC has been found to temporarily disrupt water holding capacity in O_a - A_b horizon soil pores as a function of short-term hydrophobicity (Mikita-Barbato et al., 2015). However, we suspect long-term moisture holding capacity over the length of the study increases as carbonates from anthropogenic PyC mineralize, resulting in increased total P sorption (Chintala et al., 2014) and C coalescence into pools (Preston and Schmidt, 2006). Researchers find forest soils react quickly to PyC dispersal based on substantial soil surface area (Sigmund et al., 2017)—exponentially greater than bare soil—which accommodates greater nutrient uptake and improves soil moisture retention (Licht et al., 2017). Where wildfire is no longer experienced, such as in coastal sand plain pitch pine forests (Lee et al., 2018), and prescribed fire is employed, there is a need to determine if alternative sources may be found to replicate positive impacts of prescribed fire PyC. One prominent benefit is ecophysiological; it results from nutrient mobilization and moisture retention. As an increase in photosynthetic intrinsic water use efficiency or $iWUE$ (Renninger et al., 2013; Carlo et al., 2016), $iWUE$ is derived from stable isotope analysis (stated as per mils: parts per thousand, notated as ‰) and quantifies the efficiency of carbon capture to water loss at the leaf level (Farquhar et al., 1989; Lajtha and Michener, 1994).

Where forest fire is absent and prescribed fire is unavailable for use, mechanical thinning is an option which fosters open canopies and reduces forest floor or grassland fuel (Anderson, 2017). This bioturbation method (Abney et al., 2019) is akin to other types of disturbance (e.g., animal, human, or climate pressures). Thinning is found to generate temporary nutrient turnover following soil disturbance (Saurasunet et al., 2018) without the associated risks of fire close to human settlement. We conduct an analysis which determines it is a much more expensive intervention (\$3,000 US/0.404 h) than prescribed fire (\$300 US/0.404 h), but may be used on a limited basis (cost estimates based on a report by US Fish and Wildlife Service, Hadley MA, USA, 2013). Indeed, tree removal and soil disturbance may be as, or more, effective at controlling pitch pine populations than prescribed fire (Lee et al., 2018).

In this paper, we examine prescribed fire and anthropogenic PyC impact on subsurface water and nutrient supply changes to pitch pine growth and $iWUE$. Our aim is to take PyC results and frame a “package” of anthropogenic PyC conservation potential for small, but critically and vitally important pitch pine populations where prescriptive fire is not an option in the Northeast U.S.

MATERIALS AND METHODS

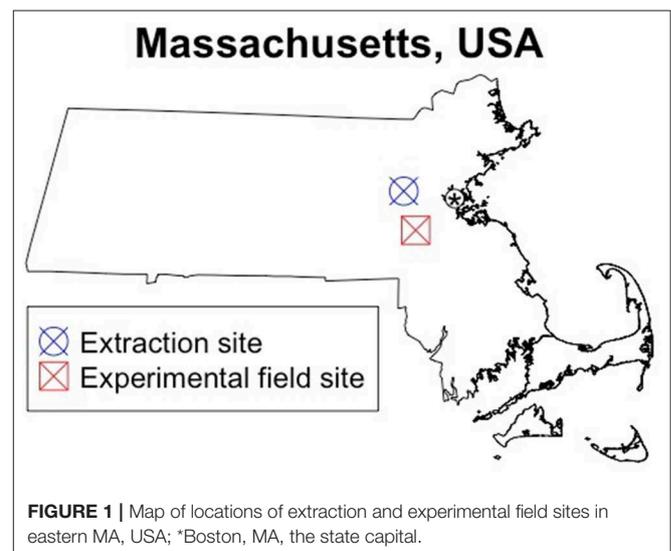
Study and Extraction Sites

A study site ($42^{\circ}39'18.23''N$ $71^{\circ}35'01.34''W$) in Middlesex County, MA was used; its environmental and meteorological characteristics are similar to those of a pitch pine community 14.5 km southeast from the forest ($42^{\circ}22'51.57''N$ $71^{\circ}28'22.60''W$) where a prescribed fire was carried out on site in the same county (Figure 1). The forest site elevation was 82 m; the experimental site elevation was 90 m.

Materials

Soil extractions from the forest were taken from the “O-A” horizon at 20 cm depth at random locations in burned and unburned portions of the 20.23 h forest using a narrow shovel. Untreated (absent charcoal) soils were characterized as Histosol (85%), Freetown (8%), and Scarboro (7%) according to NRCS SoilWeb. The dehydrated bulk density was $\pm 2 \text{ g cm}^{-3}$, estimated using the Kettler et al. (2001) rapid HMP method with 3% aqueous solution and 0.053 mm sieve to collect the sand fraction and settled silty fraction following decantation of clay followed by calculations of different fractions (clay = 3.4%, silt = 29.8%, and sand = 66.8%). Three soil treatments were created using (1) forest soil absent forest charcoal, (2) forest soil blended (9–11% v/v) with forest charcoal PyC and (3) forest soil absent charcoal blended (10% v/v) with anthropogenic PyC.

Forest charcoal PyC consists of charcoal particulate extracted from a shallow (<12 cm) burned duff and screened (<10 mm) for blending as treatment F. To determine pyrolysis temperature of the forest charcoal we conducted a fire event simulation with several pitch pine seedlings at an outdoor experiment site. Using a K-type probe (4.8 mm in diameter and 30 cm long) and data logger (EXTECH EA11) we measured a high temperature parameter where the probe was attached 88 cm above the tree base. A mean pyrolysis maximum temperature (T_{max}) between 409 and 415°C was recorded 9–12 s after exposure of the bark



to a steady flame. We ascertained these findings were well below crown recordings ($T_{\max} = 650\text{--}700^{\circ}\text{C}$, reported by A. Richardson, Harvard Forest) and ground layer (surface; $T_{\max} = 550\text{--}600^{\circ}\text{C}$ reported from an experimental site in the New Jersey pine barrens, Foereid et al., 2015).

Retort PyC (Mitchell et al., 2016; Licht et al., 2017) was produced in an Adam-style retort (Sullivan Farm, Sullivan, New Hampshire USA). To gauge production temperature, a built-in retort probe and data logger continuously recorded temperature ever 6 h (reported in Licht et al., 2017) achieving T_{\max} in the 590–615°C range. The T_{\max} results were consistent with experiments described by others (Mitchell et al., 2013; Ghorbel et al., 2015). Retort PyC was also pulverized in a small blender and screened to <10 mm particles.

Experiments

At the field site, twenty-four 32 cm depth planting holes were excavated one meter apart under an open canopy. Excavated field soil was set aside. Three soil treatments were created comprising treatments labeled: no treatment = NT, soil plus forest PyC = PyCF and soil plus anthropogenic PyC = PyCA. Eight 2.5 L pots (9.6 cm wide × 30 cm depth; CP413CH Steuwe and Sons Inc., Tangent OR USA) were filled with either 1,420 mL NT, 1,280 mL NT, and 142 mL PyCF and 1,280 mL NT and 142 mL PyCA. Thus, there were 8 pots for each treatment, a total of 24 ($N = 24$) unpooled replicates. Each pot was planted with a single 3 year pitch pine seedling (Pinelands Nursery, Princeton, NJ) and then plunged in planting holes. Seedlings were irrigated immediately after planting and left to acclimate (to stabilize according to moisture, nutrient, CEC, pH, etc.). No further irrigation was furnished during the balance of the experiments. Daytime temperatures averaged 21.66°C during the mid-day of the growing season. Fall-winter-early spring temperatures ranged from 15°C down to −13.3°C. Surface winds were variable year-round, averaging $\leq 7\text{ km h}^{-1}$. Year-round humidity averaged 56%. Average annual precipitation was 113.69 cm. As described below, soil, needle samples, and growth measurements were obtained on an annual basis for 3 years.

Field Measurements

Plant Height, Stem Diameter, and Root Length

In the fall of each year, three individuals in each treatment group were randomly selected for tree biometrics. Seedling height was measured from ground level. Primary stem diameter at 5 cm height was measured using a ProSkit electronic digital caliper (Amelia, VA, USA). Root length (along a coarse to fine gradient) was also measured. To accomplish this measurement, trees were carefully excavated and then reinstalled in each planting hole.

Laboratory Measurements

Gravimetric Moisture Retention

We performed a dry down-to-moisten up bench test resulting in a *post-hoc* analysis of extracted soil sample moisture retention. An extra set of three pots per treatment per year pots were placed in planting holes which allowed us to extract aliquots that endured the same mineralization over 3 years—after measurement the soils were returned. Identical volume samples (175 mL) were extracted and dried at 100°C for 2 days, then placed in a 300 mL

plastic cup to which 70 mL of H₂O was added. These were measured for gravimetric moisture retention on a wet basis utilizing the Shields method to conduct these experiments as in Licht et al. (2017). Net moisture retention as a subset of soil moisture evaporation (ψ_g) was determined over a 10 week period as net evaporative loss or adsorption to surfaces (Qi et al., 2018).

Carbon and Nitrogen Stable Isotopes, Elemental Analysis

Soil $\delta^{13}\text{C}$ (isotopic) measurements were acquired for five randomly selected individuals per treatment per year following extraction of three soil types each year in early summer, over a span of 3 years. Leaf $\delta^{13}\text{C}$ (isotopic) measurements were also obtained on fully expanded leaves (needle cluster) in 2015, 2016, and 2017 at peak PAR. Sample fascicles were separated and dried for 2 days at 60°C and then ground in a SPEX ball mill (Metuchen, NJ, USA). Ground samples were fed to a Perkins Elmer Elemental Analyzer ECS 4100 (Waltham, MA, USA) coupled with a Thermo Delta (Waltham, MA, USA) V+ IR-MS continuous flow isotope ratio mass spectrometer with a universal triple collector. Combustion gasses were separated on a gas chromatograph column, passed through a diluter and reference gas box, and introduced into the spectrometer. Measured $\delta^{13}\text{C}$ (Farquhar et al., 1989) was used as a proxy for photosynthetic intrinsic water use efficiency (iWUE).

Foliar Nutrient

Fifty milliliter needle samples of five randomly selected individuals per treatment per year were extracted from seedlings and dried for 2 days at 60°C. Then they were ground in a SPEX ball mill (Metuchen, NJ, USA), sieved to <10 mm, and submitted for standard plant tissue nutrient analysis using a TJA Model 975 AtomComp ICP-AES (Thermo Jarrell-Ash Corp., Franklin, MA) and Leco CN-2000 Carbon-Nitrogen Analyzer (Leco Corp., St. Joseph, MI). A standard 5 mL trace-metal-grade HNO₃ treatment was employed, then refluxed on a hotblock at 80°C for 2 h, diluted to 25 mL with 0.45 micron PTFE syringe filters and acidified using 5% HNO₃. Analysis was focused on inorganic fractions (Ca, P, K, Mg, Al, and Zn). Foliar C and N were calculated from elemental analysis.

Soil Nutrient

Repeated tests of 240 mL volume samples of untreated and treated soils were subjected to Modified Morgan digestive analysis using inductively coupled plasma spectroscopy (ICP) to measure micronutrient concentrations, proton activity of a 1:1 slurry to measure pH. The sum of extractable K⁺, Ca²⁺, Mg²⁺, and Na⁺ were used to gauge soil cation exchange capacity (CEC) measured using Formic acid (HCO₂H). Repeated analyses of 240 mL volume samples were performed to determine inorganic and organic chemistry of pyrolysis charcoals. Tests were conducted using Modified Morgan digestive analysis following the procedure of Licht et al. (2017). Due to low sample size, these data were not analyzed using the statistical methods indicated below, but results indicate that soil Ca, P, Mg, K, and Zn all tended to increase following PyC amendments (**Table 4**). Soil C and N were calculated from elemental analysis.

Statistical Analysis

We used analysis of variance (ANOVA) to examine the effect of soil type (after modification: PyCF and PyCA), year, soil type \times year on plant growth (height, stem diameter, and root length), soil water retention, and leaf foliar chemistry (% C, % N, C/N molar ratio, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$). The leaf C/N was log transformed before model fitting to meet normality assumptions. Soil water retention was arcsin square root transformed because it was percentage data. As foliar chemistry measurements were made on the same individual trees across multiple years, we used a repeated measures design using the “lme4” package in R (Bates et al., 2015). We used F-tests and χ^2 tests to test for significance in the non-repeated and repeated measure models, respectively. The F-tests were performed using the “anova” function in base

R and the χ^2 tests were performed using the “Anova” function from the “car” package in R (Fox and Weisberg, 2011). Tukey’s HSD was used for *post hoc* comparison using least square means using the “emmeans” package in R (Lenth, 2019).

RESULTS

Soil treatments PyCF and PyCA increased height in the final 2 years of treatment (soil type by year: $P = 0.001$; **Figure 2** and **Table 1**). There was a nearly 50% difference in vertical growth favoring trees subject to PyC amendment compared to controls (NT) over 3 years. This was confirmed by significant *post-hoc* Tukey’s tests for treatment effects in years 2 and 3 ($P < 0.05$ in both cases), but not year 1 ($P > 0.05$). Heights did not differ

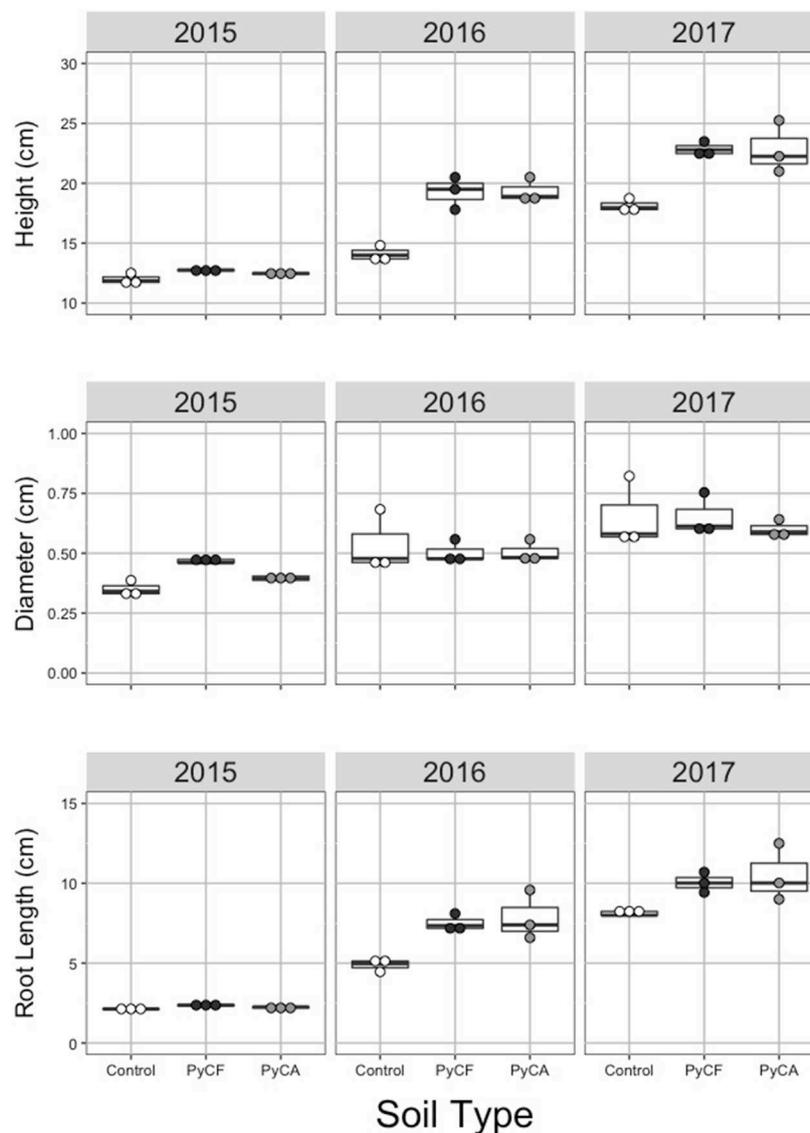


FIGURE 2 | Dot and box plots of plant height (top), diameter (middle), and root length (bottom) for soil-less media controls (white dots) and media amended with forest (PyCF; dark gray dots) and recycled feedstock (PyCA; light gray dots) pyrogenic carbon in 2015, 2016, and 2017. Statistical results can be found in **Table 1**.

between PyCA and PyCF treatments in any year (Tukey's test: $P > 0.05$). A significant treatment by year interaction ($P = 0.018$; **Figure 2** and **Table 1**) indicated that PyCF and PyCA increased root length in the second (Tukey's test: $P < 0.05$) and third (Tukey's test: $P < 0.05$), but not the first (Tukey's test: $P > 0.05$) year of the experiment. In all there was a 125% increase due to PyC involvement over the 3 years compared to controls. There was no difference in root length between PyCF and PyCA treatments in any year (Tukey's test: $P > 0.05$). Stem diameter was unaffected by the treatments in any single year ($P > 0.05$; **Figure 2** and **Table 1**), but did differ by year ($P < 0.001$).

There was a significant interaction between year and treatment on soil moisture retention capacity ($P = 0.004$; **Figure 3** and **Table 2**). *Post-hoc* Tukey's tests indicated that retention was significantly higher in the PyC amended soils as compared to the unamended soils in all years ($P < 0.05$ in all cases). In the first year, soil moisture retention was higher in PyCF than PyCA (Tukey's test: $P < 0.05$), but PyCF and PyCA had the same retention in the second and third year (Tukey's test: $P < 0.05$ in both cases).

Foliar C/N differed by year ($P = 0.024$), but was unaffected by soil treatment ($P > 0.05$) and the soil type by year interaction (P

> 0.05 ; **Figure 4** and **Table 3**). There was a significant interaction between year and soil type for % C ($P < 0.027$, **Table 3**), but *post hoc* Tukey's tests did not indicate a soil treatment effect in any of the 3 years ($P > 0.05$ in all cases). There were no significant year or soil type effects for % N ($P > 0.05$ in all cases; **Table 3**).

We found PyCF and PyCA treatments significantly increased foliar $\delta^{13}\text{C}$ across all 3 years ($P = 0.002$, **Figure 4** and **Table 3**). Results indicated increased photosynthetic iWUE (less negative $\delta^{13}\text{C}$) in those treatments compared to the NT treatment. Tukey's test found no significant difference between PyCF and PyCA treatment ($P > 0.05$). There were no significant differences in $\delta^{15}\text{N}$ across any treatment.

Due to a limited sample size ($N = 3$), soil and needle mineral data were not analyzed using the statistical methods indicated above, but mean and SD values are reported. Results indicate soil and foliar Ca, P, Mg, K, and Zn tended to increase following PyC amendments (**Table 4**).

DISCUSSION

Our study provides what we understand is the first comprehensive analysis of dual PyC effects on soil moisture, pitch pine seedling growth and iWUE as a function of subsurface implementation using a planting hole technique. In all, we examined root depth, seedling height, stem diameter, soil

TABLE 1 | Results from analysis of variance (ANOVA) for plant height, stem diameter and root length.

	Height (cm)			Stem Diameter (cm)		Root length (cm)	
	df	F	P	F	P	F	P
Soil type	2	46.96	<0.001	1.30	0.300	19.37	<0.001
Year	2	259.43	<0.001	27.91	<0.001	538.53	<0.001
Soil type * Year	4	7.37	0.001	1.59	0.220	3.94	0.018
Residuals	18						

TABLE 2 | Results from analysis of variance (ANOVA) for soil water retention.

Water retention	df	F	P
Soil type	2	2306.61	<0.001
Year	2	869.82	<0.001
Soil type * Year	4	5.54	0.004
Residuals	18		

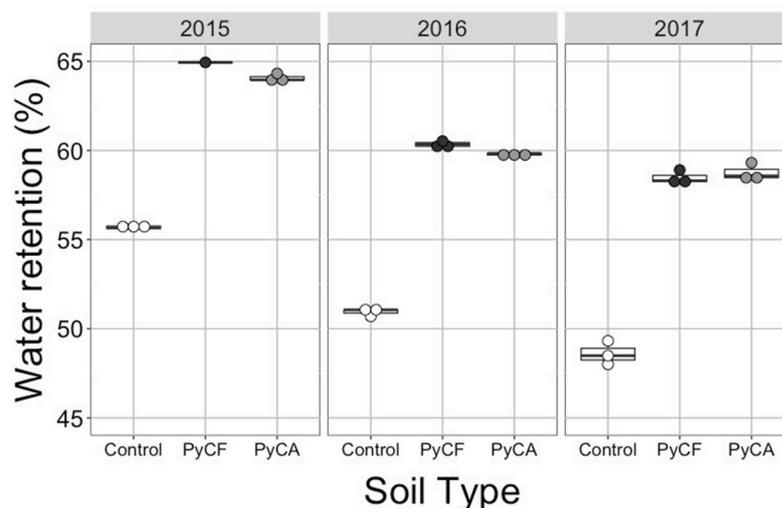


FIGURE 3 | Dot and box plots of soil moisture retention for soil-less media controls (white dots) and media amended with forest (PyCF; dark gray dots) and recycled feedstock (PyCA; light gray dots) pyrogenic carbon in 2015, 2016, and 2017. Statistical results can be found in **Table 2**.

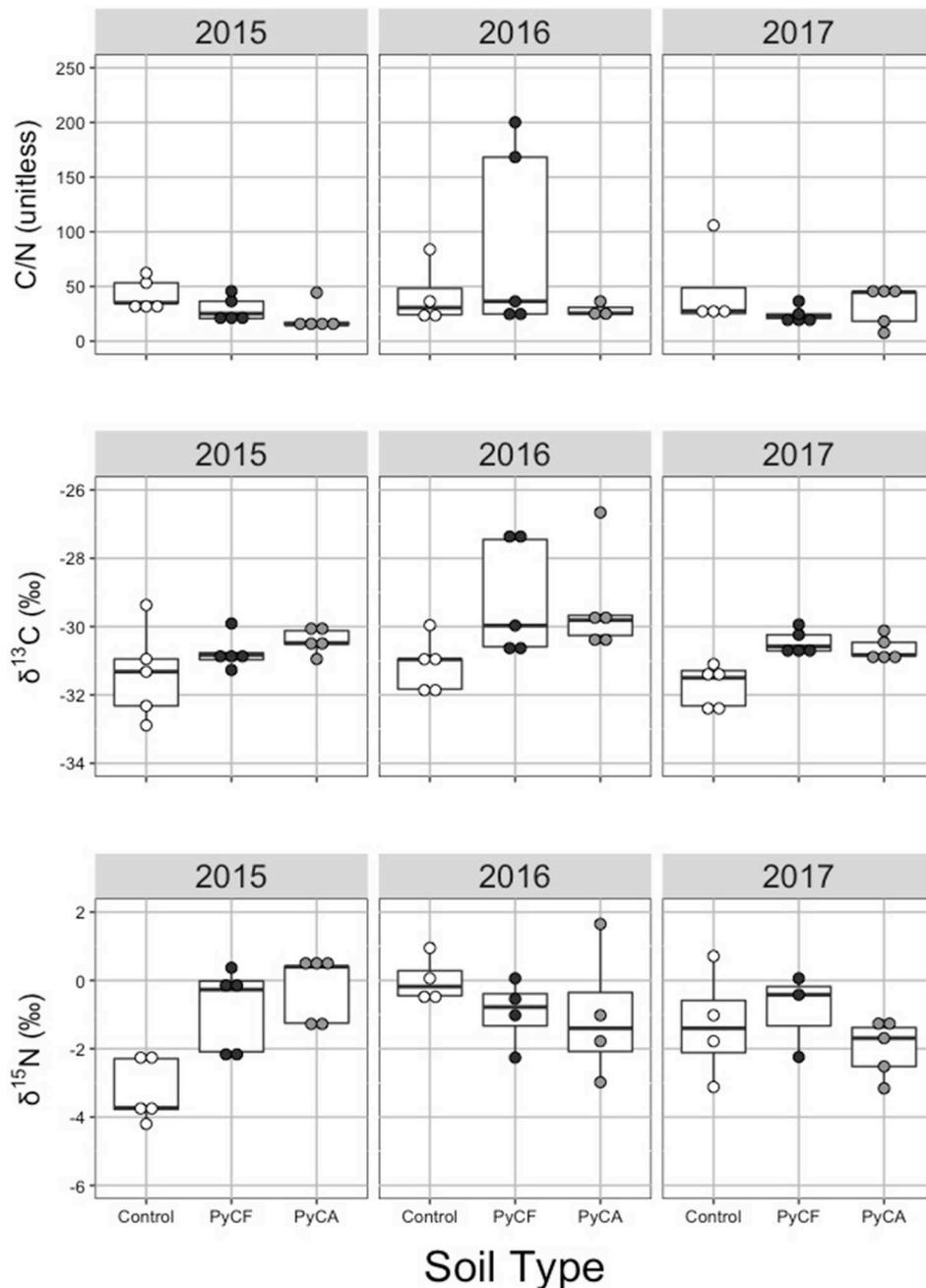


FIGURE 4 | Dot and box plots of leaf carbon to nitrogen ratios (C/N; top), ^{13}C discrimination ($\delta^{13}\text{C}$; middle), and ^{15}N discrimination ($\delta^{15}\text{N}$; bottom) for soil-less media controls (white dots) and media amended with forest (PyCF; dark gray dots) and recycled feedstock (PyCA; light gray dots) pyrogenic carbon in 2015, 2016, and 2017. Statistical results can be found in **Table 3**.

moisture retention, nutrient sorption and photosynthetic intrinsic water use efficiency at a site free of overstory and controlled resource competition. This investigation considers effectiveness of PyC intervention as the first step toward side-by-side planting hole PyC tests of pitch pine and their locust, spruce, white pine and red pine competitors. In doing so, we aspire to establish a theoretical basis for testing transferability

of this “technology” to other species which may face decline owing to absence of fire or prescribed fire. We speculate future experiments could involve jack pine (*Pinus banksiana*) with which pitch pine sometimes overlap sympatrically in the Great Lakes region, Maine and Canada.

The scope of this investigation is limited to seedlings, not regeneration (Lee et al., 2018) or germination studies but at

TABLE 3 | Results from the mixed model analysis of variance for leaf carbon to nitrogen ratios (C/N), ¹³C discrimination (δ¹³C), and ¹⁵N discrimination (δ¹⁵N).

Foliar analysis	df	Leaf C/N		Leaf %C		Leaf %N		Leaf δ ¹³ C		Leaf δ ¹⁵ N	
		χ ²	P	χ ²	P	χ ²	P	F	P	F	P
Soil type	2	0.63	0.730	2.46	0.292	0.47	0.789	12.21	0.002	2.47	0.291
Year	2	7.47	0.024	4.66	0.097	1.63	0.443	12.42	0.002	7.60	0.022
Soil type * Year	4	5.42	0.247	10.93	0.027	2.86	0.581	2.85	0.583	19.13	<0.001

TABLE 4 | PyC, Pitch pine forest soil, Sedgemoor forest soil, and Foliar Nutrient data were extracted, analyzed, and reported between 2015 and 2017 using mean ± SD.

	mg kg ⁻¹						meq 100 g ⁻¹	-log[H ⁺]
	Ca	K	Mg	P	Al	Zn	CEC	pH
PYROGENIC CARBON SOURCE								
PyCF 2015	611	424	118	22.7	22	12	15.7	4.7
PyCA 2015	699	67	88	9.9	97	24.9	14.5	5.3
PITCH PINE FOREST SOIL								
Control 2015-2017	322.6 ± 110	41.7 ± 15	26.4 ± 11	3.2 ± 1	318 ± 115	5.2 ± 0	14.8 ± 1	5 ± 0
PyCF 2015-2017	347 ± 129	47 ± 13	46.6 ± 17	2.7 ± 1	188.7 ± 28	7.2 ± 1	13.4 ± 2	5.6 ± 0
PyCA 2015-2017	900 ± 356	46.3 ± 18	78.3 ± 23	5.5 ± 1	145.3 ± 22	11.7 ± 3	13.3 ± 1	5.20 ± 0
SEDGE FOREST SOIL								
SM 2017	162	36	14	6	76	1.3	9.4	4.9
SMPyCA 2017	277	73	36	8.4	59	1.5	9.2	5.1
FOLIAR NUTRIENT								
Control 2015-2017	2220 ± 816	2016.6 ± 782	686 ± 213	883.3 ± 326	481 ± 121	48.5 ± 18		
PyCF 2015-2017	2273.4 ± 724	3843.4 ± 1128	1276.6 ± 471	1040 ± 314	398.3 ± 214	55.6 ± 21		
PyCA 2015-2017	2326.7 ± 351	4410 ± 1450	1270 ± 428	1306.6 ± 412	500.3 ± 226	65.4 ± 29		

PyCF, forest charcoal; PyCA, retort charcoal; SM, sedge meadow soil; SMPyCA, sedge meadow soil amended with PyCA.

termination of the study we obtained results which allowed us to speculate as to what will transpire when these 6 year-old trees are more mature (say 10 to 15 years old). A previous model (Meigs et al., 2011) provides direction as to how one might scale up PyC application in mixed forest tests. We considered the cost for an application in a predominantly pitch pine forest. For example, for a 0.404h area planted with 400 pitch pine seedlings (on 3 m centers) the authors assume an anthropogenic PyC production cost of approximately \$105 (production and shipping)—about a third the cost of prescribed fire activity in the same space (based on estimates derived from International Biochar Initiative, Sonoma, CA, USA). A follow-on investigation of PyC influence on pitch pine germination could contrast broadcast methods (suprasurface vs. subsurface dispersal into O_a-A_b horizons) as well as the extent to which germination numbers increase in masticated or unmasticated soils (subject to treatment conditions).

We did not predict a soil moisture gradient change over 3 years; those results were consistent with previously reported findings that moisture retention stabilizes during pyromineral-organic decomposition (Licht et al., 2017). We reason part of the rationale for this premise rests on assumptions that sandy and perhaps gravelly soils found along the Eastern U.S. coast treated with PyC produce a similar reaction in

carbonate sorption properties leading to a concomitant reduction in hydrophobicity (Alcañiz et al., 2018). As a function of post-settlement stabilization (irrespective of PyC source), we determine increased solubility of remaining carbonates leads eventually to uptake of other minerals as reported earlier (Licht et al., 2017). Questions still arise however as to PyC influence on disruptions in moisture or nutrients (Gaskin et al., 2007) tied to over-resistance to thermal and microbial decomposition (Spokas, 2010). It is accepted by those studying PyC effects that C decomposition and disaggregation is spurred by P changes occurring well after initial soil incubation. Likewise, when molar ratios change, C and N pools may combine to defeat anisohydric, or drought stress, effects (Parker et al., 2001). Part of this response likely stems from greater C and N volatilization (Doerr et al., 2018) from forest fire or prescribed fire more than anthropogenic charcoals; we contemplate this may be explained by differences in temperature and pyrolytic duration. Previous investigation suggests foliar P is found in juvenile foliar tissue following forest fire (Schaffers, 2002). In our study, we found increases in foliar P in seedlings exposed specifically to PyCA. Another potential difference could be ascribed to a greater overabundance of N₂ (Aber et al., 1989) which is not consumed in retort pyrolysis vs. fire pyrolysis—however all of these speculations lack experimental confirmation. Unfortunately, due to a limitation in

mineral loss/gain findings, a congruent set of implications for PyC mechanics eluded us.

Past research examined production (T_{max}) temperature (which affects condensation of volatile compounds) and air temperature, but did not report oxygen availability (Santin et al., 2017), which might explain differences in sorption properties (Jindo et al., 2012). If future research determines differences between feedstocks are not a major hurdle to building a plausible methodology, this would further indemnify investment in research of PyC soil management in forest ecology and conservation. Despite these upbeat assumptions, there are admittedly obstacles to implementation based on dissimilarities in PyC feedstock and production methods on the one hand and differences between anthropogenic and forest PyC effects in a given ecosystem. Allowing for the enigmatic nature of char feedstock characteristics, we were presented with another impediment to evolving a lucid, linear connection between feedstock selection, production, post-installation effects and mechanics (Mia et al., 2017).

It was easier to assign an ecophysiological value to PyC amendment. We found a more modest increase (about 12%) in iWUE fostered by PyCF or PyCA treatments than other investigators (Carlo et al., 2016), who reported a 22% increase in pitch pine photosynthetic iWUE based on PyCF alone. There are bound to be anomalies which arise between PyC analytes for a considerable number of reasons. However, if we extrapolate from the Carlo study, we may intuit higher iWUE (lower negative) resulted from greater soil moisture holding capacity or a considerable difference in tree maturity, as just two explanations. Our dual iWUE results were similar to those in another study (Renninger et al., 2013) which supports the concept that both PyCs we studied yield similar influence on tree physiology. Unfortunately, due to cost constraints, we were unable to add more replicates to this aspect of the research; however the statistical analyses used are robust to low sample size.

Reports of pitch pine response following mechanical thinning indicate scarification increases seedling recovery as a function of soil mineral turnover (North et al., 2015; Lee et al., 2018). Thus, a future key matchup between forest and anthropogenic PyC sorption of soil nutrients should comprise a comparison of disturbance (unrelated to PyC) vs. non-disturbance. It should be acknowledged that although mineral deposits are exposed following mechanical disturbance, one caveat to the thinning method is an associated problem with soil compaction (Zisa et al., 1980).

Some research has shown a relation between forest charcoal-imbued soil and ectomycorrhizal response (Warnock et al., 2007). Early findings (Hobbie et al., 1999) suggest fire effects sway pitch pine root fungi aggregation. The function of soil microbes (Nannipieri et al., 2003), in relation to pitch pine hosts, is discussed according to N-fixing bacteria found inside the fungi mantle (Güerena et al., 2013). In the case of prescribed fire, fungal responses to repeated high burn temperature and post-burn hydrophobia (Williams and Jackson, 2007) also add to what is known about mycorrhizae presence. Indeed, advances in the study of connections between prescribed fire and fungi continue (Luo et al., 2017) along with a search for genetic similarities between disjunct pine populations.

Whether or not fire proves to be a long-term solution to southern pine beetle persistence (Lesk et al., 2017), it would be very useful to identify whether forest or prescribed fire play a decisive role in slowing acceleration of blue stain fungi aggregation (*Ophiostoma minus*) associated with southern pine beetle (Arango-Velez et al., 2018). Following such a study, if results warranted, an analysis of anthropogenic PyC inputs might also be advantageous. Up until the past decade, northward southern pine beetle migration was limited to New Jersey, Long Island NY, Hudson River Valley, and Southeastern MA. Now warming winter temperatures, coupled with the loss of mite predators, forecast greater southern pine beetle aggregation in pitch pine populations in upper New York State and the Northern tier of New England (Lesk et al., 2017). It could be PyC plays a role in plant defense against southern pine beetle given its contributions to higher resistance to drought (Licht and Smith, 2018) but there is no evidence yet that PyC reduces pathogens (Nowak et al., 2015).

Recent climate models anticipate effects of climate change on a host of coastal tree species (Fernandez et al., 2015). Nevertheless, given a paucity of knowledge about PyC mechanics, it is difficult to ascertain the extent to which PyC may be an underutilized resource in the quest for resolving environmental problems and anthropogenic perturbations. It does appear, in the case of pitch pine, at least, PyC elevates productivity where stress is minimal or curbs stress in preference for adding biomass.

Population density is a limitation on prescribed fire if not an antecedent to use of anthropogenic PyC. In New England many fires are set purposefully to reduce forest fuel compared to New York or New Jersey, but total acres consumed by this process are double the rate for states further north (Melvin, 2012). In the Southeast (Chiodi et al., 2018) and Southwest US (Neary et al., 2017), many millions of acres not burned by forest fires require controlled burn management. In these regions exhibiting drier climates than those in the Northeast, forest fire and prescribed fire combine, not just to reduce competition from other tree species, but to combat pest aggression, either southern pine beetle or western pine beetle (*Dendroctonus brevicomis* LeConte), aided by selective “spot” cutting. Rather than pests or disease, ongoing concerns about the destructiveness of forest fire in Australia (McCaw, 2013), in Spain grasslands (Úbeda et al., 2005) and in other parts of Europe (Lázaro and Montiel, 2010) grow even as efforts continue to employ prescribed fire where human population is less dense.

In this investigation we enterprise preliminary evidence for anthropogenic PyC use based on transformation of lignocellulosic and hemicellulosic wastes which could be achieved economically in vast areas of the world. We intend for our data to signal the potential for novel soil amendment, inclusive of waste conversion, to fit within an armamentarium of tools to resolve conservation and land use management concerns associated with climate warming and irregular storm events.

DATA AVAILABILITY STATEMENT

All data used in this article can be found at: https://github.com/SmithEcophysLab/pitchpine_pyc/releases/tag/v1.0 (doi: 10.5281/zenodo.3600547).

AUTHOR CONTRIBUTIONS

JL and NS conceived the work, contributed substantially to the interpretation of the data and to drafting the manuscript, gave final approval of the version submitted, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. JL carried out the field experimentation, conducted preparation for all tests analyzed at laboratories at Boston University, University of Massachusetts, and University of Maine. NS was responsible

for conducting statistical analyses and formulating figures and tables.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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