Functional traits and physiology in two New England pine species

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Abstract
Globally, plants contribute significantly to the status of many ecosystems. As anthropogenic influences threaten to alter large scale ecosystem function, understanding individual species is imperative. Plant functional traits and physiological activity play crucial roles in predicting large scale ecosystem alterations through the use of models. This study aims to contribute to the ability to predict anthropogenic impacts by using physiology and leaf/root traits to assess the adaptations of *P. strobus* (white pine) and *P. rigida* (pitch pine). Leaf level flux measurements showed pitch pine seedlings to be more active than white pine seedlings during the fall. An investment of more area of leaf or root length per unit of mass, along with higher organic matter in soil of origin, suggests that pitch pines are more productive and advantageous than white pines in this region. The results of this study adds to published studies that indicate pitch pines may be better adapted for a future world.

Introduction
Forest ecosystems, covering more than $4.1 \times 10^9$ hectares of global land area, account for a significant portion of terrestrial ecosystem services (Dixon et al., 1994). In the northern hemisphere alone, forest vegetation sequesters more than 600 million metric tons of carbon per year (Gough et al., 2008). Plant productivity and decomposition shape many ecosystem functions—including oxygen production, nutrient uptake, and transpiration—and drive important biogeochemical cycles (i.e. carbon and nitrogen), supporting many organisms including plants, animals, fungi, and microbes (Arber and Melillo, 1991, Wright et al., 2004). Since a forest’s ability to sequester carbon can be determined by its community composition, growing season length, and local climate patterns, anthropogenic climate change has the potential to alter the vital ecosystem functions of forest ecosystems (Gough et al., 2008). Changing environmental conditions, such as increasing mean temperatures and increasing atmospheric carbon dioxide (CO₂) concentrations, can induce physiological responses in plants that may result in alterations of ecosystem functions (Smith and Dukes, 2013, Cramer et al., 2001, Iverson et al., 2004). The way that plants respond to these changes in terms of adaptation and potential range shifts will shape future ecosystem function and, consequently, the provision of ecosystem services.
A major concern for the future is the ability of plants to adapt to changing climate regimes to ensure their survival. Different climate change scenarios have predicted changes in the distributions of varying forest types and vegetation throughout the United States (Iverson et al., 2004, Iverson and Prasad, 1998). Since plants play such a vital role, it is essential that we examine individual species to predict and prevent undesired changes through models and management practices. Pines are distributed widely throughout the northern hemisphere. Eastern white pine (*Pinus strobus*) and pitch pine (*Pinus rigida*) cover similar ranges and exhibit differences in abundance in New England (Iverson et al., 2007). Their importance is emphasized by their success in fire-prone landscapes, presence in a variety of environments, and association with early successional stages in response to disturbance (Keely, 2012, Ledig and Little, 1979). As tolerant plants and evergreen trees, it is valuable to study these two common northern pine species in order to understand and predict how they may respond to a changing environment.

The physiology (photosynthesis, respiration, and transpiration), traits (leaf mass per area, specific leaf area, specific root length), and stoichiometric composition of a plant (carbon and nitrogen content) are guiding factors in understanding the interactions between a plant and its environment (Reich et al., 1998, Díez et al., 1997). Measures of plant gas exchange indicate carbon assimilation and efflux, water use, and can suggest energy supply and demand. The functional traits and elemental composition of a plant can suggest the role that it plays in the cycling of carbon and nitrogen and can be used to better understand its fluxes and functions. The nitrogen content of the leaves or needles of trees is closely related to the photosynthetic capacity of a leaf or needle, since a significant amount of leaf nitrogen is invested in photosynthetic machinery (Evans 1989).

This study aims to contribute vital information on plant function and adaptation of *P. strobus* and *P. rigida* in order to better understand potential response to anthropogenic impacts through physiology and leaf/root traits. The physiological activity, physical traits, elemental composition, and responses to elevated levels of CO₂ of white and pitch pine seedlings will hopefully provide insight into the relationship between plant function, leaf nutrient allocation, and climate. Their physiological capacity and functional traits may be linked to differences between the species in regional abundance within New England. Considering that thicker needles and darker coloring may serve as speculative indicators of photosynthetic machinery and nitrogen content (Hanba et
al., 1999 and Richardson et al, 2002), we may expect to see larger fluxes and higher nitrogen content in pitch pine needles. Based on superficial observations (an initial hardiness judgement), we may expect pitch pines to be better adapted for environmental changes.

Methods

Field collection of plants and growth conditions:

Twenty-four total seedlings were collected, half of which were white pines and half of which were pitch pines. The white pines were collected from Wendell, MA and the pitch pines were collected from the Crane Wildlife Management Area (CWMA) in Cape Cod. All trees were potted in identical pots. Four seedlings of each species were potted in well mixed soil (NT) from the CWMA. Four seedlings of each species were subjected to a sandy soil treatment (ST) composed of well mixed 50% CWMA soil and 50% washed sand from the local beach (Stony Beach). The sandy soil treatment reflects physically unstable soil conditions and results in lower water retention; which offers an indication of the potential impact of a drought treatment on these species. Four of each species were left outside in potting soil. The white pine seedlings left outside were collected half way through the experiment from a more sun-exposed growth environment than the others. The sixteen seedlings, eight of each species, that were not left outside were placed within a temperature and light controlled chamber. The trees were each watered with 200 ml of water every 4 days, starting on the fifth day of being in the chamber.

Growth chamber environment

The chamber was set to a 12 hour day and 12 hour night cycle. The temperature was set to reflect an average of fall Cape Cod daytime and nighttime temperatures. The daytime temperature within the chamber was set to 15°C and the nighttime temperature was set to 12°C. The light level, or photosynthetically active radiation (PAR), within the chamber remained the same for

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1 The original experiment was intended to observe the impact that warming would have on the seedlings. After taking my first set of Li-Cor measurements at the end of the first week I changed the daytime temperature within the chamber to 25°C and the nighttime temperature to 22°C. As I was taking my second set of measurements after roughly a week of warming, I became aware of the alarming lack of activity of my trees. My advisor and I decided to drop the warming aspect of the project and simply continue to measure and observe the traits that were not influenced by the activity (or lack thereof) of the seedlings. The needles began to brown during the second week and only continued to worsen despite increased watering.
each day cycle and was set to 500 µmol photons m$^{-2}$s$^{-1}$. The lights were turned off for the duration of the night cycles.

*Gas Exchange Physiology*

After a week of placing the seedlings in the chamber photosynthetic activity, respiration and transpiration of all the seedlings were measured using the LI-6400 XT Portable Photosynthesis System. A few needles were selected, enough to cover the total area (6 cm$^2$) of the chamber head by laying them as flat as possible. If the needles measured did not cover the total area, then the area covered was measured using a ruler and was later used to adjust any measurements. The readings were taken in the following order: 1) a photosynthesis and transpiration reading with chamber settings of 1500 PAR (µmol m$^{-2}$ s$^{-1}$), 400 parts per million (ppm) CO$_2$, 20° C block temperature, and a relative humidity near or over 50% (preferably 60%) 2) a respiration and transpiration reading with the lamp turned off, so PAR = 0 µmol m$^{-2}$ s$^{-1}$, 400 ppm CO$_2$, 20° C block temperature, and a relative humidity near or over 50% 3) a photosynthesis and transpiration reading with 1500 PAR, 1500 ppm CO$_2$, 20° C block temperature, and a relative humidity near or over 50% 4) a respiration and transpiration reading with the lamp turned off, 1500 ppm CO$_2$, 20° C block temperature, and a relative humidity near or over 50%. After the readings were stable (after at least 5 minutes) between measurements 4 readings were logged for each set of measurements, each 20 seconds apart.

*Functional Traits*

Tree height, needle area, needle length, leaf dry and wet weight, fine root dry and wet mass, and root length were measured for each seedling. Twelve needles were removed from each seedling, measured for length, weighed (all together), scanned using a LI-3100 Area Meter to measure the sum of their area, dried in an oven for about 46 hours at 60° C, and weighed again. Twelve additional needles of older age were removed from each indoor pitch pine seedling (directly from the stem) and processed in the same way. Four needles were removed from each outdoor pitch pine seedling, compiled for a bulk needle sample, and processed in the same way. Fine roots were removed from each seedling, weighed, scanned using a Epson Perfection V300 Photo scanner to later measure root length using the image processing program Image J, dried in an oven for about 46 hours at 60° C, and weighed again. Additional
bulk fine root samples were taken from the four outdoor pitch pines and the four outdoor (sunny-grown) white pines and processed in the same way.

**Elemental Composition**

All dried root and needle samples along with two soil samples from each seedling collection site were ground using a WIG-L-BUG Amalgamator (a mortar and pestle for the soil). These samples were then carefully packed (5-9 mg for plant tissue and 30-40 mg for mineral soil) and processed using a NC soil analyzer (consult the MBL SES 2015 CHN analysis protocol for detailed instructions).

**Results**

*Leaf-level Physiology*

Measurements of photosynthesis and respiration and a calculation of gross photosynthesis show that pitch pine seedlings had, on average, higher fluxes of CO$_2$ than white pine seedlings (Fig. 1 and Fig. 2). We see no clear trend when analyzing the difference between the sandy treatment and no treatment across both species. For pitch pine only, however, there are higher fluxes (both photosynthesis and respiration) of CO$_2$ in the seedlings under the sandy treatment. There is no clear trend when analyzing the difference between exposure to ambient (400 ppm) and elevated (1500 ppm) CO$_2$ across the two species. However, considering pitch pine under both treatments, there are higher fluxes of CO$_2$ in and out of the needles under elevated CO$_2$.

Measurements of transpiration (water loss from stomata due to vapor pressure deficit) show that pitch pine seedlings had average higher fluxes of water (H$_2$O) than white pine seedlings (Fig. 3). Across both species there is higher transpiration under exposure to ambient CO$_2$ than elevated CO$_2$. Determination of water-use efficiency values (the ratio of instantaneous net assimilation of CO$_2$ to water loss) showed that, across both treatments and species, water use efficiency was higher under exposure to elevated CO$_2$ (Fig. 4). Carbon-use efficiency, which is calculated by __________, shows a similar trend, but with a smaller difference between the efficiency of the seedlings under exposure to ambient and elevated CO$_2$ (Fig. 5).

*Functional Traits*
Pitch pine seedlings of both treatments were found to have lower leaf mass per area (LMA) values than white pine seedlings (Fig. 6). Specific leaf area (SLA) - the inverse of LMA – was also lower in pitch pine than the white pine seedlings across both treatments. They were also found to have higher specific root length (fine root length/fine root dry mass) values than white pine seedlings across both treatments (Fig. 7).

**Elemental Composition**

Needle carbon and nitrogen content did not differ significantly between species, but all seedlings had almost 50 times higher carbon (around 50%) than nitrogen content (around 1.5%) in their needles (Fig. 8). The nitrogen content of the soil from the CWMA (pitch pine) did not differ greatly from that of Wendell, MA (white pine), however; the CWMA soil was found to have higher carbon content (Fig. 9). Carbon to nitrogen ratios from both species and treatments show that ratios do not differ greatly between species or treatments for the needles, but when comparing needle ratios to root ratios we see overall higher carbon to nitrogen ratios in the roots (Fig. 10).

**Correlations between needle traits, fluxes and composition**

There was no clear trend found in the relationship between assimilation and area-based nitrogen content of needles (Fig. 11) but a clear positive linear trend was found in the relationship between LMA and area-based nitrogen content of needles (Fig. 12).

**Discussion**

**Differential responses of physiology in two pines**

Flux measurements (assimilation, respiration, and transpiration) suggest that pitch pine seedlings were generally more active than white pine seedlings during the late fall season (Fig 1 and Fig 3). Considering that the white pine seedlings were removed from central Massachusetts, where seasonal cooling begins earlier and tends to be greater than on the cape, we can speculate that the white pines simply acclimate to cold weather (thus entering a dormant state for the winter)
earlier than the pitch pines. The white pines were also removed from Wendell over a week prior to the pitch pine removal from the CWMA and were re-potted twice (rather than once) prior to the start of the experiment; all of which could have damaged their roots and, consequently, limited their activity. As we might expect, exposure to elevated CO$_2$ appears to increase net assimilation rates for both species (Fig. 2). These rates were measured within the span of 5 minutes so they do not indicate acclimation to increased CO$_2$ but do indicate immediate responses to higher CO$_2$ concentrations within the LI-6400 chamber head. Studies have shown that elevated CO$_2$ stimulates carbon assimilation in plants as well as improving water relations by reducing transpiration, therefore increasing water-use efficiency (Prior et al. 2011, Morrison 1993). Results from this study reflect these findings (Fig.2-4). Although we might expect the pitch pine seedlings under the sandy treatment to have reduced assimilation and transpiration rates due to induced water stress that is not the case. This may be due to the fact that moisture content did not differ greatly between the two treatments (Table 1). The pitch pine seedlings belonging to the sandy treatment may also have been in better condition initially in terms of root damage or conservation of fine roots post removal from their origin, allowing them to have greater water uptake and higher assimilation rates (Fig. 13).

Functional Traits

White pine seedlings appear to invest more leaf mass per unit of dry mass than pitch pine seedlings, as illustrated by LMA values (Fig. 6). Although pitch pine needles appear bigger and to contain more photosynthetic machinery, white pine needles are actually more compact, and invest more mass per area of leaf and having more or equal amounts of machinery when expressed on an area basis (Fig. 11 and Fig 12). Literature indicates that species with low LMA (or high SLA) are associated with higher photosynthetic capacity (Reich et al. 1998). Therefore, both flux measurements and LMA values suggest that pitch pine seedlings are more photosynthetically active than white pine seedlings. Pitch pine seedlings were also found to have higher specific root lengths, meaning that they build more root length for a given dry-mass; which allows them to cover more soil area (Fig. 7). This advantage enables pitch pine seedlings to have higher rates of nutrient and water uptake (per dry mass).
Elemental composition

Since the pitch pines had higher flux rates, and photosynthetic capacity is associated with leaf nitrogen (Reich et al. 1998), we might expect the pitch pine seedlings to have higher nitrogen content. Interestingly, the two species did not differ greatly in their carbon and nitrogen contents (Fig. 8), this may signify that, in this case, environmental conditions such as seasonal changes and leaf structure may control activity more so than the amount of photosynthetic machinery present. The carbon and nitrogen content of the soils from which the two species were removed differed significantly (the CWMA having higher carbon content), showing that the pitch pine seedlings originated from an organic matter rich soil (Fig. 9). A soil rich in organic matter is indicative of higher productivity, as more productivity is required to have soil rich in organic matter. Therefore, we may expect the pitch pine seedlings to be more productive than the white pine seedlings, based only on the soil from their original locations.

Implications

Based on the data confirming higher leaf-level flux rates and increased values of SLA and SRA, pitch pines were more active in the fall compared to white pines. This may indicate that they can be productive for longer periods during the year and thus better adapted for potential seasonal changes. Although pitch pines are less abundant than white pines in New England (Fig. 14 USDA Forest Service), range and abundance models predict that white pines will experience a greater shift in abundance in response to environmental change (Fig. 15 USDA Forest Service), further supporting the implications of this study.

Although this study offers insights into the characteristics of white pines and pitch pines, further research is necessary to fully understand the responses of these species to future environments. Future research should include; responses to warmer, cooler, and carbon dioxide rich growing environments, physiological responses of adult trees, differences between in-field and controlled-setting measurements, and a survey of the functional traits of these species over a variety of growing environments (i.e. precipitation, soil conditions, and seasonality).

Acknowledgements
Many contributed to the completion of this project. Particularly my advisor, Mary Heskel and the Semester in Environmental Science staff. I would like to give a special thanks to Linda Deegan and Chris Neill for providing me with half of the seedlings for my project and much of their help when I needed it the most. Last but not least, thanks to Or Shapira, Elizabeth de la Reguera and Thomas Parker for guiding me in the right direction.
References


Meyer, B. 1928. Seasonal Variations in the Physical and Chemical Properties of the Leaves of the


Figures and Tables

Fig. 1: Means of gross photosynthesis ($A_{\text{gross}}$) values (incorporating both net assimilation and dark respiration) for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T) under exposure to both ambient and elevated CO$_2$ (400 and 1500 ppm, respectively).
Fig. 2: Means of net assimilation ($A_{net}$) and dark respiration ($R$) values for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T) under exposure to ambient and elevated CO$_2$ (400 and 1500 ppm, respectively).
Fig. 3: Means of transpiration (Trans, or water loss via stomata) values for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T) under exposure to ambient and elevated CO₂ (400 and 1500 ppm, respectively).
Fig. 4: Means of calculated values of water-use efficiency (net assimilation/water loss) for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T) under exposure to ambient and elevated CO$_2$ (400 and 1500 ppm, respectively).
Fig. 5: Means of calculated values of carbon-use efficiency (net assimilation/gross assimilation) for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T) under exposure to ambient and elevated CO₂ (400 and 1500 ppm, respectively).
Fig. 6: Mean values of leaf dry mass per area of leaf (LMA) for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T).
Fig. 7: Mean specific root length (fine root length/fine root dry mass) values for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T).
Fig. 8: Mean carbon and nitrogen content (percentage) values of needles for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T).
Fig. 9: Carbon and nitrogen content (percent) values for soil from the Crane Wildlife Management Area (CWMA) and Wendell Massachusetts from which all pitch pines and white pines were removed.
Fig. 10: Mean carbon to nitrogen ratios of the roots and needles for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T).
Fig. 11: Relationship between carbon dioxide assimilation ($A_{\text{net}}$) and area-based nitrogen content for white pine (W.P) and pitch pine (P.P) seedlings.
Fig. 12: Relationship between leaf mass per area of needles and the area-based nitrogen content of the needles for all collected white pine (W.P) and pitch pine (P.P) and seedlings. These include the pitch pine seedlings that were left outside (P.P OUT), the white pine seedlings that were collected from a sunnier spot in Wendell, MA (W.P SUNNY) and the old needles that were collected from the pitch pine seedlings kept inside (P.P Old).
Fig. 13: Images showing the roots of seedlings under the sandy treatment (left) and the no treatment (right).
Fig. 14: Maps showing the abundance of eastern white pine (left) and pitch pine (right) with circles indicating the area of interest (New England).
Fig. 15: Projected abundance changes for white pine (top) and pitch pine (bottom) under climate change scenario. The importance value is the abundance in a given forested area.
Table 1: Mean moisture readings of the top and bottom soil within pots, made a few days after fluxes were measured, for white pine (W.P) and pitch pine (P.P) seedlings of both the sandy treatment (S.T) and no treatment (N.T).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean top soil moisture (VWC)</th>
<th>Mean bottom soil moisture (VWC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.P N.T</td>
<td>13.6 ± 0.73</td>
<td>19.7 ± 1.6</td>
</tr>
<tr>
<td>P.P S.T</td>
<td>17.0 ± 0.57</td>
<td>24.2 ± 0.82</td>
</tr>
<tr>
<td>W.P N.T</td>
<td>12.9 ± 0.38</td>
<td>19.2 ± 0.60</td>
</tr>
<tr>
<td>W.P S.T</td>
<td>15.8 ± 0.70</td>
<td>22.3 ± 1.3</td>
</tr>
</tbody>
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