Ericoid mycorrhizae influence nitrogen acquisition by *Vaccinium macrocarpon* (American cranberry)

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Introduction

Vaccinium macrocarpon (American cranberry) is an important crop in New Jersey's agricultural industry, with the 2018 crop valued at $15.8 million, making New Jersey the third-largest grower of this fruit in the United States[1]. Generally, *Vaccinium* species account for a significant portion of New Jersey's total crop production value, accounting for 65% of the 2018 fruit crop valuation and 26% of the total fruit and vegetable crop valuation in that same year[2]. Beyond their agricultural value, *Vaccinium* and its relatives are also prominent members of the forest understory in the upland forests of the New Jersey Pine Barrens, especially *V. vacillans* (lowbush blueberry), *Kalmia latifolia* (mountain laurel) and *Gaylussacia baccata* (black huckleberry)[3]. In the wild, *V. macrocarpon* is commonly found in the Atlantic White Cedar bogs of New Jersey's Outer Coastal Plain[3]. These plant species and other members of family Ericaceae are adapted to the poor soil quality and high fire frequency that are characteristic of the New Jersey Pinelands region[4].

Further, mycorrhizal fungi are known to improve the fitness of plants, including *Vaccinium* species[5,6]. The formation of root associations between plants and mycorrhizal fungi is known to benefit host plants through increased capacity to acquire water and nutrients[7], as well as through increased salt tolerance[8], aid in defense of plant pathogens, and the sequestration of heavy metals[9]. Ericaceous plants form root associations with ericoid mycorrhiza (ERM), a mycorrhizal group primarily comprised of Ascomycetes[5,9]. These associations are known to benefit *Vaccinium* species in agroecosystems as well as natural environments; ERM colonization of *V. corymbosum* (highbush blueberry) increases root biomass[10], as well as fruit mass and flower abundance[11].

Unlike its host plants, ERM are able to access nitrogen from recalcitrant sources[12], through the secretion of hydrolytic enzymes[13], which decompose organic detritus and create a pool of inorganic nutrients. These nutrients can then be absorbed by fungal hyphae and shared with host plants[9]. ERM have also been found to successfully break down chitin, a complex polysaccharide, in order to procure nitrogen[14]. Further, ERM are able to facilitate nitrogen uptake by host plants, even in low pH environments[5], and presence of ERM is known to increase the efficiency of nitrogen uptake in *V. macrocarpon*[6]. Specifically, root associations formed between *V. macrocarpon* and the ERM symbiont *Rhizoschyphus ericae* increase the uptake of nitrate, a bioavailable form of nitrogen, which is essential for plant growth; nitrogen absorbed by *R. ericae* hyphae are made readily available to *V. macrocarpon* hosts[6].

Given the ecological and agricultural relevance of *V. macrocarpon* and its relatives, and the prevalent use of synthetic inorganic nitrogen as fertilizer in agricultural systems, the mechanisms of nitrogen uptake by *Vaccinium* are of particular interest. Notably, McArthur and Eaton (1989) found that excessive fertilization with inorganic nitrogen, potassium, and phosphorus decreased flowering in commercial cranberry strains. When grown in agricultural settings with well fertilized soils, *V. macrocarpon* produces low-quality leaf litter and maintains active ERM symbiosis[15]; these are also traits of *V. macrocarpon* in nutrient-poor soils. Promotion of ERM associations may therefore promote nitrogen uptake from organic sources, and limit the necessity for inorganic fertilizers in *Vaccinium* agroecosystems[16].

Because ERM are capable of obtaining nitrogen from organic sources and are known to share these nutrients with host plants, *V. macrocarpon* may preferentially utilize nutrients obtained from its mycorrhizal symbionts over freely available synthetic nitrogen fertilizers. By controlling *V. macrocarpon* exposure to mycorrhizae and providing an inorganic nitrogen fertilization treatment containing a ^{15}N isotopic tracer, we evaluate the preferred nitrogen source of *V. macrocarpon* and provide additional context to the cycling of nitrogen between *V. macrocarpon*, its mycorrhizal symbionts, and the rhizosphere. Further, we hypothesize that (1) generally, mycorrhizal presence will increase overall leaf nitrogen concentration, and (2) when inorganic fertilizers are applied, *V. macrocarpon* will preferentially uptake nitrogen from organic sources when ERM are present.

Methods

Plant Growth and Treatment

For a period of eight weeks, *V. macrocarpon* specimens were grown under 24-hour fluorescent light in a LabLine Biotronette Mark III Environmental Chamber and watered daily with 10 mL portions of distilled water. Standard potting soil was sterilized by autoclaving for 30 minutes at 250 °C before propagation. Parent plant material, a *V. macrocarpon* vine of the "Crimson Queen" variety, was provided by the Philip E. Marucci Center for Blueberry and Cranberry Research.

Figure 1. *Vaccinium macrocarpon* specimens growing during experimental treatment stage.

After six weeks, 25 plants were transferred to individual 550 mL planters and filled with approximately 250 g of soil (Figure 1). The soil utilized at this stage had a sandy consistency and was sourced from a region of the Brendan T. Byrne State Forest, in Burlington County, New Jersey, known to have a high abundance of ericaceous plants, including *V. vacillans* and *G. baccata*. Although the same soil was provided to all 25 plants, 14 plants received sterilized soil, in order to prevent the formation of mycorrhizal associations (Fig. 2). The remaining 11 plants received untreated soil as a means of natural ERM inoculation (Fig. 2). This method of ERM inoculation has been shown to successfully initiate mycorrhizal colonization of container-grown plants[17]. Plants were allowed two weeks to acclimate to these new conditions, under 24-hour fluorescent light, with daily watering.

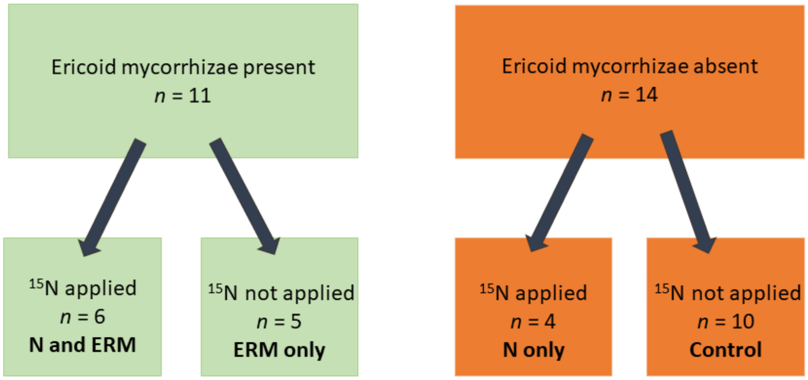

Figure 2. Experimental Design. Bold text in lower boxes indicates name of associated treatment group with reference to presence of ericoid mycorrhizae (ERM) and addition of inorganic nitrogen (N).

Supplemental nitrogen fertilization began 10 weeks after initial propagation. At this time, all plants were moved near a window, so as to provide natural light and establish a light-dark cycle. For 12 consecutive days, four non-mycorrhizal plants and six mycorrhizal plants were fertilized with the daily application of 100 microliters of a 150 mg/L ^{15}N ammonium nitrate solution (Figure 2).

Elemental Analysis

Upon conclusion of the ammonium nitrate treatment, isotopic analysis was performed on dried leaf material from each plant specimen, via a Thermo Delta V isotope ratio mass spectrometer, interfaced to a NC2500 elemental analyzer. Further elemental analysis to determine total carbon and nitrogen content of the growth medium was performed using a Thermofisher FlashSmart C:N Soil analyzer.

Statistical Analysis

Statistical analyses were conducted in R. Normality of data within each treatment group (Figure 2) was assessed using the Shapiro-Wilk Test for Normality. Two-way Analysis of Variance (ANOVA) evaluated differences in isotopic nitrogen ratio ($^{15}N:^{14}N$), total leaf nitrogen, and total leaf carbon, using mycorrhizal presence/absence and presence/absence of ^{15}N fertilizer as predictor variables. Post-hoc testing was conducted with Tukey's Honest Difference Significance Test. To compare the quality of the growth medium between treatment groups, total soil carbon and total organic matter for each treatment group were compared to that of the control group using two-way ANOVA.

Results

Compared to control plants, total soil carbon did not vary significantly in plants that received the nitrogen fertilization treatment ($p > 0.05$) (Figure 3). However, plants that formed ERM associations but did not receive nitrogen fertilization had significantly greater soil carbon when compared to control plants ($p < 0.05$) (Figure 3).

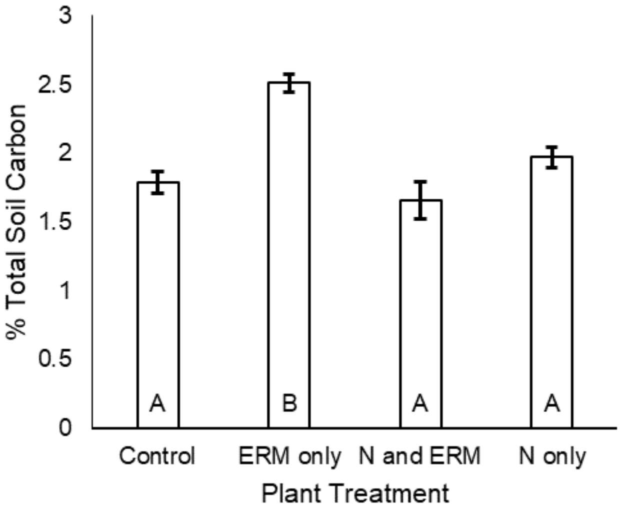

Figure 3. Total carbon, expressed as a percentage, of soil used as experimental growth medium. Letters denote results of two-way ANOVA ($p < 0.05$).

Based on statistical analysis, presence of ERM did not influence the ratio between ^{15}N and ^{14}N (hereinafter referred to as “isotopic nitrogen ratio”) ($p > 0.1$), but nitrogen fertilization did have a significant effect on this response ($p < 0.001$) (Figure 4). The combined effect of ERM associations with nitrogen fertilization did not significantly affect isotopic nitrogen ratio ($p > 0.1$) (Figure 4).

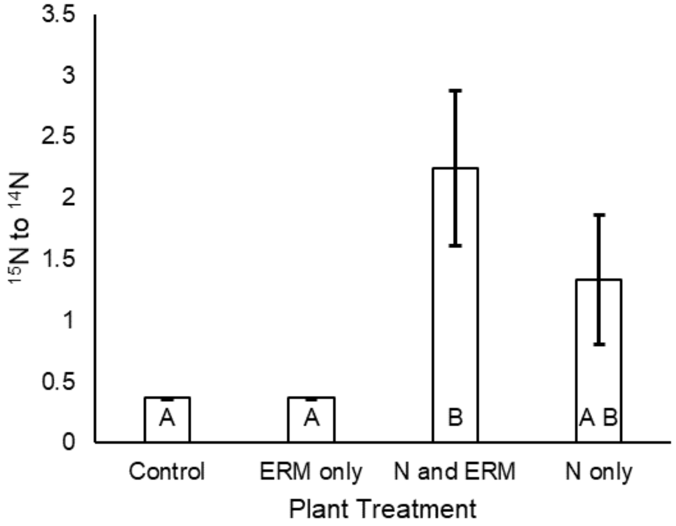

Figure 4. Ratio of nitrogen isotopes (^{15}N to ^{14}N) observed in *V. macrocarpon* leaf samples following isotopic analysis. Letters denote results of two-way ANOVA ($p < 0.05$).

Plants that received ^{15}N fertilization in the absence of ERM did not differ significantly in their isotopic nitrogen ratio from those that did not receive this treatment ($p > 0.05$) (Figure 4). This represents the baseline ^{15}N:^{14}N ratio. Furthermore, plants with mycorrhizal associations in the presence of ^{15}N fertilization did exhibit significant increases in isotopic nitrogen ratio, when compared to control plants ($p < 0.05$), but this did not differ significantly from that of fertilized plants without ERM ($p > 0.05$) (Figure 4). If mycorrhizal plants treated with ^{15}N accessed more inorganic nitrogen, as hypothesized, the "N only" treatment group would have had a greater isotopic ratio than the "N and ERM" treatment group. However, the reverse is true, and ERM plants exhibit a slightly better rate of acquisition of inorganic N than non-mycorrhizal plants (Figure 4).

Although ERM presence did not significantly affect isotopic nitrogen ratio, it did increase overall nitrogen uptake when compared to non-mycorrhizal treatment groups ($p < 0.05$) Total leaf carbon uptake was increased in ^{15}N-fertilized mycorrhizal plants, compared to control plants ($p < 0.05$).

Discussion

Because ericoid mycorrhizal fungi are able to obtain nitrogen from organic sources, and are known to increase nitrogen uptake by the roots of their host plants, it was hypothesized that nitrogen utilized by *Vaccinium macrocarpon*, an ericaceous vine, would predominantly come from organic sources in the presence of ERM, even after the application of inorganic nitrogen fertilizer. However, the results of this study suggest that *V. macrocarpon* and its mycorrhizal symbionts do not preferentially absorb nitrogen derived from organic sources over inorganic sources, as hypothesized. Instead, inorganic materials were the preferred nitrogen source, given the observed increase in isotopic nitrogen ratio in fertilized ERM plants (Figure 4).

Further, fertilized ERM plants were also found to acquire a greater amount of nitrogen than if ERM were not present: ^{15}N uptake was greater on average in the mycorrhizal treatment group than in the non-mycorrhizal treatment group, although this increase was not statistically significant (Figure 4). As anticipated, ERM presence did significantly increase overall leaf nitrogen content, regardless of inorganic fertilization, corroborating results of previous studies that suggest ericoid mycorrhizae mobilize nitrogen from rooting zones and share these resources with host plants, specifically with *Vaccinium macrocarpon*[6,15].

Analysis of the growth medium indicates that the ERM-only treatment group was exposed to a greater portion of organic matter during the growth period, with a greater portion of soil carbon (Figure 3) than other treatment groups, but this increase was likely not sufficient to influence nitrogen uptake.

Future research will include a larger sample size and standardization of organic matter across all treatment groups. Further, because nitrogen dosage in this study was standardized within fertilized treatment groups, but not across all four treatment groups, it is difficult to quantify the total acquisition of nitrogen from organic sources that occurred during the growth period. Follow-up research should standardize the nitrogen content of the organic material, using leaf litter tagged with ^{15}N to trace nitrogen from organic sources. Further, ERM colonization within treatment groups was not quantified. Although non-mycorrhizal plants received only sterilized soil, it is possible that some colonization did occur and influence the results shown here. To resolve this, future research should also quantify ERM presence/absence in the growth medium.

This study suggests that *V. macrocarpon* does not prefer organic sources of nitrogen over inorganic sources, which contributes to a greater scientific understanding of the relationship between *Vaccinium macrocarpon* and ERM. The significant differences in nitrogen uptake between mycorrhizal and non-mycorrhizal *V. macrocarpon* individuals corroborate earlier research that container-grown ericaceous plants can be effectively colonized with natural mycorrhizal inoculum[17]. In accordance with established knowledge of mycorrhizal function[5,6,9], nitrogen content was significantly increased in mycorrhizal plants compared to non-mycorrhizal plants. This result furthers the understanding that mycorrhizal associations are of benefit to plant hosts, particularly with respect to nutrient acquisition.

Given the ecological and economic significance of ericaceous plants and their mycorrhizae, continued research should work to further elucidate the nitrogen consumption habits of this symbiotic partnership. Because this research did not confirm that preference of mycorrhizae influences the origin of nitrogen utilized by *V. macrocarpon*, these hypotheses should be tested again under more robust conditions.

Acknowledgements

The authors would like to thank the Phillip E. Marucci Center, Dr. Nicholi Vorsa, and Jennifer Johnson-Cicalese for their provision of plant material and guidance during the propagation process. The authors would also like to acknowledge the Rutgers University-Camden Dean's Undergraduate Research Grant, which funded this work, and thank Dr. Mike Gallagher, Steve Schulze, Dr. Jennifer Oberle, and Alexis Everland for their assistance in the lab and suggestions during the development of this report.

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