Plant Physiol. Rep. (July–September 2019) 24(3):410–421
https://doi.org/10.1007/s40502-019-00459-7

ORIGINAL ARTICLE

Effects of irrigation and phosphorus fertilization on physiology, growth, and nitrogen-accumulation of Scotch broom (*Cytisus scoparius*)

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Received: 9 July 2019 / Accepted: 21 September 2019 / Published online: 30 September 2019

Abstract We tested the effects of phosphorus (P) fertilization and soil water on the growth, physiology, and total nitrogen (N) accumulation in N-fixing Scotch broom in Olympia, WA. We manipulated soil water and P availability via irrigation and fertilization, respectively, in a completely randomized 2 × 2 factorial on potted one-year old Scotch broom seedlings (n = 20) in an N-deficient sand. There was substantial evidence that increased-irrigation and P-fertilization had similar positive effects on N accumulation in Scotch broom approximately equally. High-irrigation rates were more often associated with positive physiological and growth responses in Scotch broom than fertilization, however. Although the irrigation × fertilization interaction was not significant, there were additive effects of high-irrigation and fertilization on biomass and N content as both were 50% greater in the fertilized-and-high-irrigation treatment relative to the respective fertilized and high-irrigation treatments. We noted an accumulation of N and P in the plant tissues. Analyses indicated a pattern of decreasing function and growth with increasing N and P concentrations in Scotch broom biomass, suggesting plant growth and physiology were limited by some other resource. Total plant N content values ranged from 7.0 ± 1.1 g plant^{-1} in the control and 23.4 g ± 9.0 plant^{-1} in the fertilized-and-high-irrigation treatment. Extrapolated to typical densities of comparably sized Scotch broom plants on invaded sites in the western Pacific Northwest, these findings suggest that, at least, 12–65 kg N ha^{-1} would be found in Scotch broom plants in the field.

Keywords Soil water · Transpiration · N-fixation · Biomass · Water-use efficiency

Introduction

A nitrogen-(N) fixing shrub native to the Mediterranean (Tutin et al. 1968), Scotch broom is a ubiquitous invasive species, today found on six continents (Potter et al. 2009). A rapid-grower well-adapted to drought and nutrient limitations (Williams 1981) and a prolific producer of seed, Scotch broom can readily out-compete native species (Fogarty and Facelli 1999) and dominate sites (Haubensak and Parker 2004). Scotch broom is particularly an issue in the western Pacific Northwest, USA, where it is estimated to be the most economically costly invasive plant in the forests of the region (Mefford et al. 2017). The degree to which certain edaphic factors mediate N-accumulation in Scotch broom and how N influences its growth and physiology is still unclear. Understanding this would elucidate the impact Scotch broom can have on a system, in terms of N-inputs, and the importance of N-fixation in Scotch broom's competitive advantage over native species.

Studies investigating the impact of Scotch broom invasion on soil properties and nutrient cycling have found

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increases in soil N following invasion (Wheeler et al. 1987; Fogarty and Facelli 1999; Haubensak and Parker 2004; Caldwell 2006; Grove et al. 2015), whereas others have reported no change in N (Shaben and Myers 2009; Carter et al. 2018). The change in soil N under Scotch broom may be site-specific (Slesak et al. 2016). The ability of Scotch broom to fix as much as 100 kg N ha^{-1} $year^{-1}$ (Barros et al. 2012) and potentially retain this N until it dies has led to research studying the use of Scotch broom plant material as an organic fertilizer (Domínguez et al. 2018). Ecologically, potential N inputs of Scotch broom need to be measured as resulting changes in the cycling of nutrients caused by this invasive has the potential to alter the availability of nutrients and disrupt ecosystem function (Prober and Lunt 2009).

Terrestrial biological nitrogen fixation (BNF) in N-fixing plants is regulated, in part, by environmental factors. N-fixing plants have been found to have higher nutrient demands than non-fixers; specifically, N-fixing plants have been found to require greater quantities of carbon (C), phosphorus (P), sulfur, magnesium, and, due to the requirements of the N-fixing enzyme nitrogenase, molybdenum and iron (Evans et al. 1993; Schulze 2004; Hedin et al. 2005; Barron et al. 2009; Vitousek et al. 2013). The availability of plant metabolites can regulate N-fixation as *Rhizobia* are dependent on photosynthate supply from the host plant for energy (Sprent 1985).

Most factors potentially limiting to BNF, however, are considered indirectly related to low soil water availability (Marino et al. 2007). Bhuvaneswari et al. (1981) found that *Rhizobia* were unable to infect hosts when drought prevented plants from actively growing root hairs: the infection site for *Rhizobia*. Similarly, because of the metabolic costs of forming a symbiosis with *Rhizobia*, plants will actively down-regulate nodulation under stress conditions (Guerin et al. 1990).

Less is known, however, about P as a regulator of N-fixation. Scotch broom has been associated with reductions in soil P pools (Caldwell 2006; Slesak et al. 2016) and P has been shown to have a strong effect on Scotch broom growth (Williams 1981; Rivaie 2011). In addition to being an essential macronutrient for plant growth and function, P is critical for N-fixation—although its role in the process remains unclear. In general, P is a component of ATP (adenosine triphosphate) and, at least, 16 ATP molecules are hydrolyzed for each molecule of N_2 reduced (Berg et al. 2002). Higher relative concentrations of P in nodules compared to other plant organs and a preferential supply of P to nodules in recovering, P-limited environments have been found (Israel 1993). Conversely, Eaglesham and Ayanaba (1984) submit down-regulation of BNF is due to the indirect effect of P deficiencies leading to reduced legume growth. Houlton et al. (2008) posited that N-fixers use high amounts of N to produce phosphatase to release bioavailable P back to the plant for fueling N-fixation.

Relatively few studies have investigated the factors regulating N accumulation in Scotch broom (e.g., Helgerson et al. 1984; Watt et al. 2003a) and many questions remain. We could find no studies that measured the amount of above- and belowground N content in Scotch broom and how these quantities may be affected by resource availability. In order to understand the impacts this widespread invasive To address this knowledge gap, we planted Scotch broom seedlings (n = 20) in pots using N-deficient sand as the soil medium in a completely randomized 2 × 2 factorial with P-fertilization and irrigation. We sought to (i) quantify the relative degree to which P and soil water influence N content in Scotch broom and (ii) relate the quantities of N in Scotch broom with their physiology and growth.

Methods

This study took place at the Olympia Forestry Sciences Laboratory (OFSL) in Olympia, Washington, USA. Average annual precipitation is 1470 mm $year^{-1}$ (2012–2017) and average monthly precipitation during the growing season (April–September; 2012–2017) is 101, 59, 32, 9, 27, and 68 mm, respectively (WSU AgWeatherNet—Tumwater SW). During the study period, total precipitation was 1478 mm in 2016 and 1659 mm in 2017. Precipitation during the growing season months (April–September) in 2016 was 45, 6, 37, 18, 16, and 56 mm (April and May were drier than usual) and 147, 88, 39, 2, 4, and 36 mm in 2017.

The Scotch broom seedlings used in the study originated from seeds collected from the Olympia area. The seeds were sown and germinated in a greenhouse in 2015, and germinants were buried in raised beds to overwinter. In April of 2016, 20 seedlings of comparable size and vigor were selected and planted into individual 151.4-L (76.2 cm × 76.2 cm × 51.1 cm) pots and placed in a fenced-in raised-bed study area. The pots were filled with washed river sand with virtually no available N (0.008% ± 0.01). By using a soil medium with very low N, the majority of N within the plant and the soil at the end of study could be reasonably assumed to be atmospherically sourced through fixation by Scotch broom.

Ten of these seedlings were randomly selected to receive a high irrigation treatment and the other 10 received a low irrigation treatment (high irrigation = 3500 mL per day; low irrigation = 700 mL every other day). The irrigation treatments began in mid-May to coincide with the onset of the summer drought. The low-irrigation rate was set to mimic a low severity drought:

18 mm of precipitation plus ambient per month. The high-irrigation treatment was set to be non-limiting: 180 mm of precipitation plus ambient per month. The treatments were delivered using a programmable timer using plastic irrigation tubing with variable-flow emitters attached at the end of the tubes.

Ten broom plants, five randomly selected within each of the two irrigation treatments, had 12.8 g of triple-super phosphate (5.8 g of P) spread evenly over the soil surface at the beginning of each growing season, thus doubling the P content (34.1 mg P kg^{-1}) by mass relative to a local site near Matlock, WA having similar soil texture and high P availability (15.3–18.7 mg P kg^{-1} soil; Slesak et al. 2016). The four treatments in this study were therefore: (1) low-irrigation and non-fertilized (hereafter "control"), (2) fertilized, (3) high-irrigation, and (4) high-irrigation and fertilized.

The variability in *Rhizobia* present in the soil among pots was reduced using a *Rhizobia* inoculate mixture. Nodules (9.6 g) were harvested from the root mass of eleven mature, local Scotch broom plants within 1 km of the OFSL. Once removed, the nodules were submerged in 500 mL of water and ground with a pestle. The resulting inoculant mixture was then applied using a pipette at a rate of 15 mL per plant at the base of each stem in June of 2016.

Data collection

Soil moisture sensors (model EC-5, METER Group, Inc., Pullman WA, USA) were installed horizontally at 30 cm depth in each of the 20 pots. Volumetric soil water content (SWC) was recorded at an hourly interval each day throughout the growing season with an Em50 data-logger (METER Group, Inc., Pullman WA, USA).

Using a LI-COR 6400XT portable infrared gas analyzer, we measured transpiration rates (mmol H_2O m^{-2} s^{-1}), photosynthetic rates (μmol CO_2 m^{-2} s^{-1}), and water-use efficiency rates (WUE; μmol CO_2 m^{-2} s^{-1} mmol H_2O m^{-2} s^{-1}) across treatments. Measurements were taken periodically during the growing season (five dates in 2016 and four dates in 2017), once in the morning (0800–1030 h PDT) and again in the afternoon (1300–1530 h PDT). The frequency of measurements was intended to provide a robust mean of seasonal physiological function. A reference CO_2 level of 400 ppm was used for all measurements. A PAR of 500 (μmol m^{-2} s^{-1}) was used in the morning and a PAR of 1500 was used in the afternoon. Foliar samples used to measure physiology did not fill the standard leaf chamber of the LI-COR 6400 XT. Leaf area measurements of sampled portions of Scotch broom were made by processing digital images of the samples in ImageJ (Rueden et al. 2017). These measurements were then used to calibrate the area-based physiological measurements.

Measurements were taken to monitor root collar diameter (mm), height (based on height of tallest stem; cm), crown width (cm), and crown volume (m^3). Crown width was estimated by measuring the diameter of the crown in both the north-south and east-west cardinal directions.

Height and the two crown widths were converted to canopy volume (m^3) using the equation from Thorne et al. (2002):

$$CV1 = 2/3\pi h(a/2 * b/2); \quad (1)$$

where *CV*, crown volume (m^3); h, height; a, north-south crown width, and b, east-west crown width.

Soil samples were collected at the end of the experiment using a soil probe at two depths (0–25 cm and 25–50 cm) at three locations within a 10 cm radius from the stem of the plant. Samples from a given depth were composited, air-dried, sieved to pass a 2 mm mesh, and archived prior to chemical analysis. Total soil C and N were measured on a 1-g pulverized subsample with dry combustion using a LECO Dumas combustion technique on a Fisons NA1500 NCS Elemental Analyzer (ThermoQuest Italia, Milan, Italy). Available soil P was estimated using the Bray extraction followed by calorimetric estimation of P on a spectrophotometer (Spectronic 20 Genesys, Model 4001, Thermo Electron Corporation).

After two growing seasons, all Scotch broom plants were excavated and roots were washed. Belowground and aboveground biomass were separated at the root collar, dried (at 65 °C until constant weight was achieved) and weighed to compare above- and belowground biomass allocation across treatments. Above- and belowground samples were then ground to < 1 mm in a Wiley mill for additional analyses. Two belowground samples from the control treatment were damaged during excavation, reducing the sample size from five to three. A dry-ash procedure was performed on a subsample of all belowground samples to account for any sand present in the sample after washing.

Ground above- and belowground biomass samples were analyzed for total C, N, and P concentrations using dry combustion with an Elementar (Elementar Analysensysteme GmbH). Prior to analysis, the belowground biomass samples were processed using a density separation procedure to remove any sand contaminant in the sample. The process involved using a high-density liquid solution (sodium polytungstate) at a density of 1.7 g cm^{-3}. This was shaken for 1 h. The organic matter sample was then physically skimmed off of the top of the liquid, dried and analyzed. All nutrient concentrations were reported on a dry matter basis.

To assess all fractions of total N in the artificial potted systems of this study, leachate was periodically quantified. Leachate, as it pertains to this study, was defined as water extracted from the bottom of the pots under slight tension. The collection of leachate occurred once during the months of November, January, and March. A TOC/TN-TOC-VCSH machine (Shimazu Company) was used to estimate total N per leachate sample using a dry combustion method (720 °C).

Models predicting soil and plant C, N, P, growth, and biomass were fit using the gls function in the nlme package (Pinheiro et al. 2015) in R v 3.4.0 (R Core Team 2017). Data with repeated measurements were analyzed using a first-order auto-regressive correlation structure for repeated-measures ANOVAs. Predictor variables were fertilization and irrigation. Post-hoc comparisons were made using the least squares means (LS means) function in the lsmeans package (Lenth 2016). Comparisons were made within date when significant. *P* values were adjusted using the Tukey method to avoid spurious results. The statistical significance threshold was $\alpha = 0.1$ for all analyses due to the inherently high variability in the variables measured in this study. Means reported throughout are followed by their standard errors.

To test for differences in leachate total N (mg L^{-1}), the main effect of date (November, January, and March) was added as a predictor. Since soil C, N, and P were analyzed from samples collected at two depths (0–25 cm and 25–50 cm), the random error term was designated as depth nested within pot.

Treatment effects on aboveground biomass, belowground biomass, total biomass, aboveground:belowground biomass ratio, and relative growth rates were assessed. Belowground biomass was log-transformed to meet assumptions of normality and homoscedasticity. Two belowground biomass samples were excessively damaged during excavation and were omitted from the dataset.

Nutrient concentrations of the plant tissue and soil were analyzed to estimate differences in the relative amount of N fixed and P assimilated. Concentrations of C (%) and N (%) and P (mg kg^{-1}) in the above- and belowground biomasses were multiplied by the appropriate plant biomass variables to estimate total elemental content per plant (above-, belowground, and total). These C, N, and P content values were also analyzed as C:N and N:P. Belowground biomass, plant tissue and soil nutrient concentration response variables were log-transformed to meet assumptions of normality and homoscedasticity.

Relative growth rates (RGR) of basal diameter, height, and crown volume of Scotch broom between measurements—which ranged from 1 week to approximately 2 weeks—were also analyzed. Relative growth rates were calculated using the following equation:

$$\mathrm{RGR} = (\ln M_2 - \ln M_1)/(t_2 - t_1) \quad (2)$$

RGR is the relative growth rate expressed as an estimate of proportionate daily growth. t_2 is time two or the later date and t_1 is time one or the earlier date. M_2 and M_1 are the natural logarithmically transformed broom size measurements taken during t_2 and t_1, respectively.

During the biomass harvesting, eleven of the twenty plants were found to have small diameter roots emerging out of the pots and into the soil of the raised beds. To account for this artifact, diameters of the root cross-sections were measured and converted to total root cross-sectional area (cm^2) per pot. The mean of the total root cross-sectional area varied among treatments as follows: control: 0.11 ± 0.05 cm^2 (emergent roots present in all five pots), fertilized: 0.31 ± 0.17 cm^2 (emergent roots present in four of the five pots); high-irrigation: 0.0 ± 0.0 cm^2 (emergent roots present in none of the pots); fertilized-and-high-irrigation: 0.22 ± 0.21 cm^2 (emergent roots present in two of the five pots). Since the presence and size of the roots were influenced by treatment (i.e., greater frequency and size in the low irrigation treatment), total root cross-sectional area was converted to an adjusted residual covariate using the methods from Huitema (2011; "quasi-ANCOVA"). This adjusted residual covariate was then used in analyses to account for its influence on all response variables. A quasi-ANCOVA is similar to ANCOVA except that the residuals of the ANOVA model applied to the covariate are used in place of values for the covariate. Using the residuals eliminates bias in the adjusted means from the ANCOVA that would otherwise be introduced when treatments affect a covariate.

N and P concentrations in the above- and belowground biomasses were used to predict mean physiological rates, growth rates (mm $year^{-1}$ and cm $year^{-1}$), and biomass per plant in linear regression analyses. Soil water usage was summarized using bi-weekly (instead of daily, as above) soil water depletion. Absolute bi-weekly soil water content was analyzed using the maximum SWC recorded for a given sensor over the two growing seasons (a surrogate for pot-specific soil saturation) as a covariate. This covariate accounts for any inherent pot-level variation in soil water holding capacities and sensor installation. Absolute growth rates were calculated by subtracting the initial growth measurement from the final growth metric measurement and dividing by two, to get a rate of growth over the two growing seasons. Absolute growth rates and biomass were log-transformed to meet assumptions of normality and homoscedasticity.

Results

Biomass

Irrigation and fertilization significantly affected Scotch broom aboveground biomass (Table 1). The high-irrigation treatment had significantly greater aboveground biomass than the low-irrigation treatment and the fertilized treatment had greater aboveground biomass than the non-fertilized treatment. Conversely, only irrigation was significant in predicting belowground and total biomass. In both cases, the high-irrigation treatment had significantly greater biomass than the low-irrigation treatment. No explanatory variables were significant in predicting aboveground:belowground biomass ratio.

Plant nutrient content

Aboveground, belowground, and total N content were significantly influenced by irrigation and fertilization (Table 2). The high-irrigation treatment had significantly greater aboveground N content than the low irrigation treatment and the fertilized treatment had significantly greater aboveground N content than the non-fertilized. The N content in belowground biomass was significantly greater in the high-irrigation than the low-irrigation treatment. Again, a similar trend was seen belowground with fertilization as the fertilized treatment had greater belowground N content than the non-fertilized. Taken together, total N content was significantly greater in the high-irrigation treatment than in the low-irrigation treatment, and total N content in the fertilized treatment was significantly greater than in the non-fertilized treatment.

The irrigation × fertilization interaction significantly influenced aboveground P content and total P content (Table 2). The fertilized-and-high-irrigation treatment had significantly greater aboveground P content than the control, fertilized, and high-irrigation treatments. While the fertilized-and-high-irrigation treatment had significantly greater total P content than the control, fertilized, and high-irrigation treatments.

The main effects of irrigation and fertilization (Table 2) were significantly affected by belowground P content. The high-irrigation treatment had significantly greater belowground P content than the low-irrigation treatment. Similarly, the fertilized treatment had significantly greater belowground P content than the non-fertilized treatment.

The main effects of irrigation and fertilization (Table 2) significantly influenced belowground C:N only. The low-irrigation and the non-fertilized treatments had greater C:N than the high-irrigation and the fertilized treatments, respectively. There were no effects of treatments on C:N in the aboveground content, nor C:N in the total biomass content.

The irrigation × fertilization interaction significantly influenced the aboveground and total biomass N:P. The high-irrigation treatment had the greatest N:P in the aboveground and total biomasses among treatments.

Soil nutrient concentrations and leachate

The irrigation × fertilization interaction significantly influenced soil N concentration (data not shown). The high-irrigation treatment ($< 0.0001 \pm 0.001\%$ N) had lower soil N concentration than all other treatments (control = 0.003 ± 0.002; fertilized = 0.010 ± 0.003; fertilized-and-high-irrigation = 0.007 ± 0.02; pre-treatment percent N = $0.008 \pm 0.01\%$).

The fertilization × depth interaction significantly influenced soil P concentration. The soil in the 0–25 cm depth in the fertilized treatment had greater P (17.3 ± 2.4 mg kg^{-1} P) than all other treatment and depth combinations (non-fertilized × 0–25 cm = 3.3 ± 0.2 mg kg^{-1} P; non-fertilized × 25–50 cm = 3.9 ± 0.4 mg kg^{-1} P; fertilized × 25–50 cm = 6.6 ± 0.5 mg kg^{-1} P). The fertilized × 25–50 cm had the second greatest soil P concentration and was significantly greater than the non-fertilized treatment at both depths. The two non-fertilized treatment depths did not differ significantly.

There was a significant interaction of irrigation × fertilization on total soil water N, but multiple comparisons failed to detect differences among treatments. Among the three months samples were taken, the control had the greatest mean total soil water N concentration with 0.78 ± 0.21 mg L^{-1}, followed by the fertilized-and-high-irrigation treatment (0.52 ± 0.07 mg L^{-1} N), the high-irrigation treatment (0.47 ± 0.07 mg L^{-1} N), and the fertilized treatment (0.46 ± 0.07 mg L^{-1} N).

Relative growth rates

The irrigation × fertilization × date interaction significantly influenced basal diameter RGR over the course of the study (Fig. 1). Basal diameter rates differed significantly during the August 23, 2016 measurement period. The fertilized-and-high-irrigation treatment had a lower basal diameter RGR than all other treatments and the control had a lower growth rate than the high-irrigation treatment (estimate = 0.02 ± 0.01 m m^{-3} day^{-1}).

The irrigation × date and the fertilization × date significantly affected crown volume RGR (Fig. 2). The irrigation treatment had a significantly greater RGR during the first measurement period, June 21, 2016 (estimate = 0.04 ± 0.01 m^3 m^{-3} day^{-1}). The fertilized

Table 1 Summary of Scotch broom biomass responses to the main effects of irrigation and P fertilization—mean ± SE in parentheses

	Number of plants	Aboveground (g)	Belowground (g)[a]	Total (g)	Abv:Blw
Main effects					
Low-irrigation	10	506.8 (137.2)	192.0 (51.4)	791.5 (199.4)	4.9 (2.2)
High-irrigation	10	**1091.4 (310.9)**	**526.0 (136.4)**	**1617.4 (424.5)**	2.7 (0.6)
Non-fertilized	10	502.3 (109.6)	241.4 (44.6)	835.2 (137.2)	2.2 (0.5)
Fertilized	10	**1095.9 (320.8)**	486.5 (146.8)	1582.4 (446.5)	2.0 (0.4)

Bold font denotes significant differences within biomass allocation totals (columns) among main effects—irrigation and fertilization

[a]Two belowground biomass samples from the control were excessively damaged during excavation and were not included in these estimates

treatment had a greater RGR than the non-fertilized treatment: RGR on June 21, 2016 (estimate = 0.01 ± 0.01 m^3 m^{-3} day^{-1}), July 12, 2016 (estimate = 0.02 ± 0.01 m^3 m^{-3} day^{-1}), and August 2, 2016 (estimate = 0.03 ± 0.01 m^3 m^{-3} day^{-1}) were each significantly greater for the fertilized treatment. This relationship reversed in 2017, with the non-fertilized treatment having a greater RGR than the fertilized treatment. The non-fertilized treatment was significantly greater on June 28, 2017 (estimate = 0.02 ± 0.01 m^3 m^{-3} day^{-1}). Height was not analyzed further as only 'date' was significant.

Relationships between N, P, Scotch broom physiology and growth

Plant N and P concentrations were rarely significant in predicting physiology and growth in Scotch broom (Table 3). Apart from transpiration rates and aboveground N concentration, the relationships between plant N and P concentrations and physiology and growth were negative when significant.

Discussion

Findings from this study suggests that soil water and P may equally limit N accumulation in Scotch broom. Analyses indicated a pattern of decreasing function and growth with increasing N and P concentrations in Scotch broom biomass. Further supported by a greater belowground C:N under non-fertilized and low-irrigation main effects, this suggests that N and P may accumulate in Scotch broom when plant growth is limited by another resource. The fertilized-and-high-irrigation treatment resulted in the greatest N accumulation, although not significantly. High-irrigation rates were more often associated with positive physiological and growth responses in Scotch broom than fertilization but, in general, increased irrigation and P-fertilization equally affected the N-content within Scotch broom.

Nutrients

Overall, N content within the plants increased under high-irrigation and fertilization to nearly equal degrees (Table 2). Although the irrigation × fertilization interaction was not significant, there were additive effects of high-irrigation and fertilization on biomass accumulation (Table 1) and N content (Table 2), with both responses showing greater than 50% increases in the fertilized-and-high-irrigation treatment relative to the fertilized and high-irrigation treatments, respectively.

While other studies have investigated the content of N in aboveground biomass of Scotch broom, we could find no studies that looked at total quantities of N within individual plants or the effects of soil water and P on total N accumulation in Scotch broom. Instead, past studies have largely examined Scotch broom communities (Wheeler et al. 1987; Watt et al. 2003b), subsampled the plant components, or used proxies (Helgerson et al. 1984; Haubensak and Parker 2004) to infer N-fixation and content. The values for aboveground N content in Scotch broom from this study can be used to estimate quantities of N contained in Scotch broom plants on an area basis. Limited N was transferred to the soil as values did not differ from the pre-treatment value of 0.008 ± 0.01%. Leachate-N collected did not differ significantly among treatments. Therefore, N content values should reflect a realistic, conservative estimate of plant tissue N, as maximum and minimum thresholds for soil water and P-limitations on N-fixation may not have been reached, and thus, N-accumulation estimates may not reflect the full range of potentials for Scotch broom.

Slesak et al. (2016) estimated a Scotch broom density of 0.22 stems m^{-2} on two sites in the western Pacific Northwest that were of comparable heights to those harvested in this study: 272 cm and 176 cm, respectively. Using the range of values found in this study, we would conservatively estimate that between 11.8 and 64.8 kg N ha^{-1} would be found in Scotch broom plants under these conditions.

Table 2 Mean ± SE of (1) aboveground, belowground, and total biomass C, N, and P content and C:N and N:P

	n	Aboveground (g)	Belowground (g)[a]	Total biomass (g)
Nitrogen				
Main effects				
Non-fertilized	10	6.2 (1.3)b	1.7 (0.3)	8.9 (1.5)b
Fertilized	10	13.7 (4.2)a	2.8 (0.9)	16.5 (5.0)a
Low-irrigation	10	6.2 (1.5)B	1.4 (0.3)B	8.7 (1.9)B
High-irrigation	10	13.7 (4.1)A	3.0 (0.8)A	16.7 (4.9)A
Treatment				
Control	5	4.4 (1.3)	1.0 (0.4)	7.0 (1.1)
Fertilized	5	8.1 (2.6)	1.6 (0.5)	9.7 (3.1)
High-irrigation	5	8.0 (2.0)	2.1 (0.4)	10.1 (2.2)
Fertilized-and-high-irrigation	5	19.4 (7.4)	4.0 (1.6)	23.4 (9.0)
Phosphorus				
Main effects				
Non-fertilized	10	4.9 (0.9)	0.5 (0.1)b	6.0 (1.0)
Fertilized	10	11.3 (3.1)	1.0 (0.3)a	12.3 (3.4)
Low-irrigation	10	5.0 (1.0)	0.3 (0.1)B	6.6 (1.2)
High-irrigation	10	10.7 (3.2)	1.1 (0.3)A	11.7 (3.5)
Treatment				
Control	5	4.4 (1.1)B	0.2 (0.1)	5.9 (1.4)B
Fertilized	5	6.5 (1.7)B	0.4 (0.1)	6.9 (1.8) B
High-irrigation	5	5.5 (1.6)B	0.6 (0.2)	6.0 (1.5)B
Fertilized-and-high-irrigation	5	16.0 (5.4)A	1.6 (0.6)	17.6 (5.9)A
C:N				
Main effects				
Non-fertilized	10	37.4 (1.7)	47.4 (7.4)a	38.4 (1.9)
Fertilized	10	37.9 (1.1)	37.8 (2.3)b	37.8 (1.0)
Low-irrigation	10	36.7 (1.6)	50.0 (6.6)A	38.7 (1.6)
High-irrigation	10	38.6 (1.2)	35.7 (2.7)B	37.6 (1.2)
Treatment				
Control	5	35.4 (2.3)	63.5 (15.2)	38.5 (4.1)
Fertilized	5	38.1 (2.2)	41.9 (3.0)	39.7 (1.6)
High-irrigation	5	39.4 (2.3)	37.7 (4.9)	39.7 (2.2)
Fertilized-and-high-irrigation	5	37.8 (1.1)	33.6 (2.6)	36.8 (1.4)
N:P				
Main effects				
Non-fertilized	10	1.3 (0.1)	5.7 (1.7)	1.6 (0.1)
Fertilized	10	1.1 (0.1)	1.2 (0.3)	1.2 (0.1)
Low-irrigation	10	1.0 (0.1)	4.8 (0.5)	1.3 (0.1)
High-irrigation	10	1.3 (0.1)	4.2 (1.4)	1.5 (0.1)
Treatment				
Control	5	1.0 (0.1)B	5.7 (1.2)	1.2 (0.1)AB
Fertilized	5	1.1 (0.2)B	4.2 (0.2)	1.3 (0.2)AB
High-irrigation	5	1.6 (0.2)A	5.7 (2.7)	1.8 (0.2)A
Fertilized-and-high-irrigation	5	1.1 (0.2)B	2.6 (0.4)	1.2 (0.2)B

Significant differences are denoted by case-sensitive letters within columns and reflect results with significant differences shown within main effects or among treatments, respectively

[a]Two belowground samples from the control treatment were excessively damaged during excavation and were removed from analyses, affecting the estimates

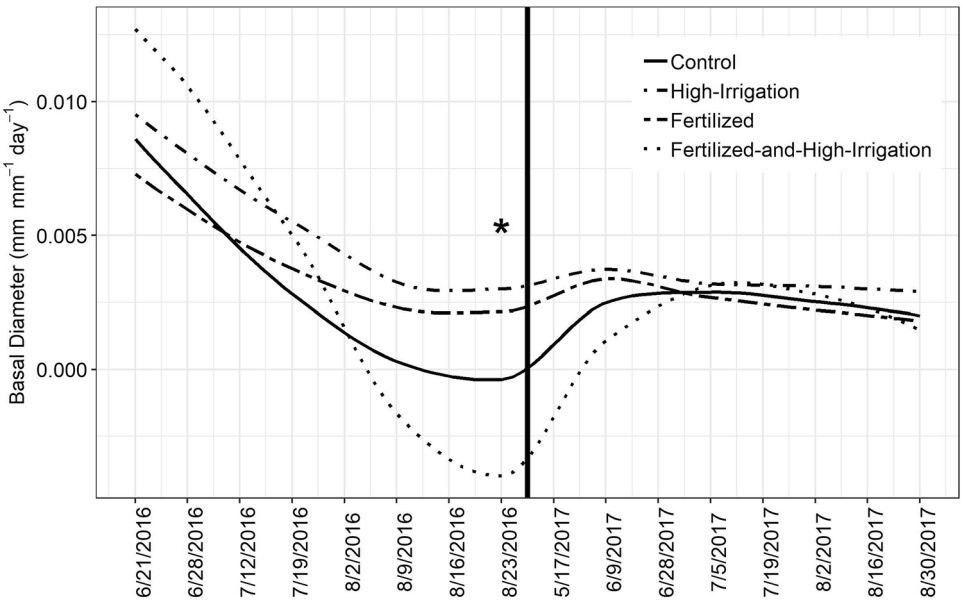

Fig. 1 Basal diameter relative growth rates (RGR). The vertical bar separates growing seasons. Asterisks denote significant differences

As low soil water availability has been documented to inhibit fine root development in legumes—the infection site for *Rhizobia* (Bhuvaneswari et al. 1981)—we expected a greater soil N and assimilated N in the high-irrigation treatment than the fertilized treatment. Although this did occur (total biomass N content: high-irrigation = 10.1 ± 2.2 g N vs. fertilized = 9.1 ± 3.1 g N), the differences were not significant. However, the high-irrigation treatment did have a significantly greater aboveground biomass N:P than all other treatments and a greater total biomass N:P than the fertilized-and-high-irrigation treatment (Table 2). This finding could be further indication that soil water has a slightly greater positive effect on N-fixation than P. It could also mean that we reached a saturating level of P in the fertilized treatments that began to yield less fixed N in the biomass per additional unit of P.

Irrigation seemed to increase uptake of P by Scotch broom in this study. The fertilized-and-high-irrigation treatment had the greatest total biomass P content (17.6 ± 5.9 g) of all the treatments in this study including the fertilization-only treatment (6.9 ± 1.8 g). We found a greater abundance of P at a greater depth in the pot in the fertilized-and-high-irrigation treatment than the fertilized-and-low-irrigation treatment. This greater vertical distribution of P likely increased its contact with plant roots and availability for uptake. The positive effect of irrigation on growth likely also increased plant demand for P.

N-fixation has been posited to be used to produce phosphatase—an enzyme which requires high amounts of N for production—to mineralize P (Houlton et al. 2008). Caldwell (2006) found this enzyme to be in greater abundance in the presence of Scotch broom. To support the hypothesis regarding the role of N in producing phosphatase, we would have expected plants to down-regulate phosphatase production and thus N-fixation. We saw the opposite trend: P-fertilization increased N content in the plants. The data, therefore, indicate that the roles of P in ATP and increasing plant growth may be more important in terms of influencing N-accumulation in these N-fixing plants.

Relating nutrient status to function and growth

N and P content were associated with greater biomass but N and P concentration in the biomass were negatively related with physiological and growth rates. N and P likely accumulated in plants that were limited by some resource (Malliard et al. 2016), causing this negative relationship. This was unexpected as responses were clearly positively influenced by treatment, although Helgerson et al. (1984) found similar results in both red alder (*Alnus rubra*) and Scotch broom. Furthermore, the lack of a relationship between percent N in aboveground biomass and C-assimilation in this study is consistent with other work examining these dynamics. Vance and Heichel (1991) posited that under non-stressed conditions, photosynthate supply to nodules does not regulate BNF. Nodules can accumulate poly-hydroxylbutyrate (Bergersen et al. 1995) and store starch (Forrest et al. 1991), indicating adequate or abundant supplies of carbon. Even under stress conditions, Fellows et al. (1987) showed high levels of sucrose in the nodules of soybeans during simulated drought conditions. At the global scale, however, N-fixing plants are predominantly found in high-light environments, presumably due to the high energy cost of N-fixation symbioses (Houlton et al. 2008).

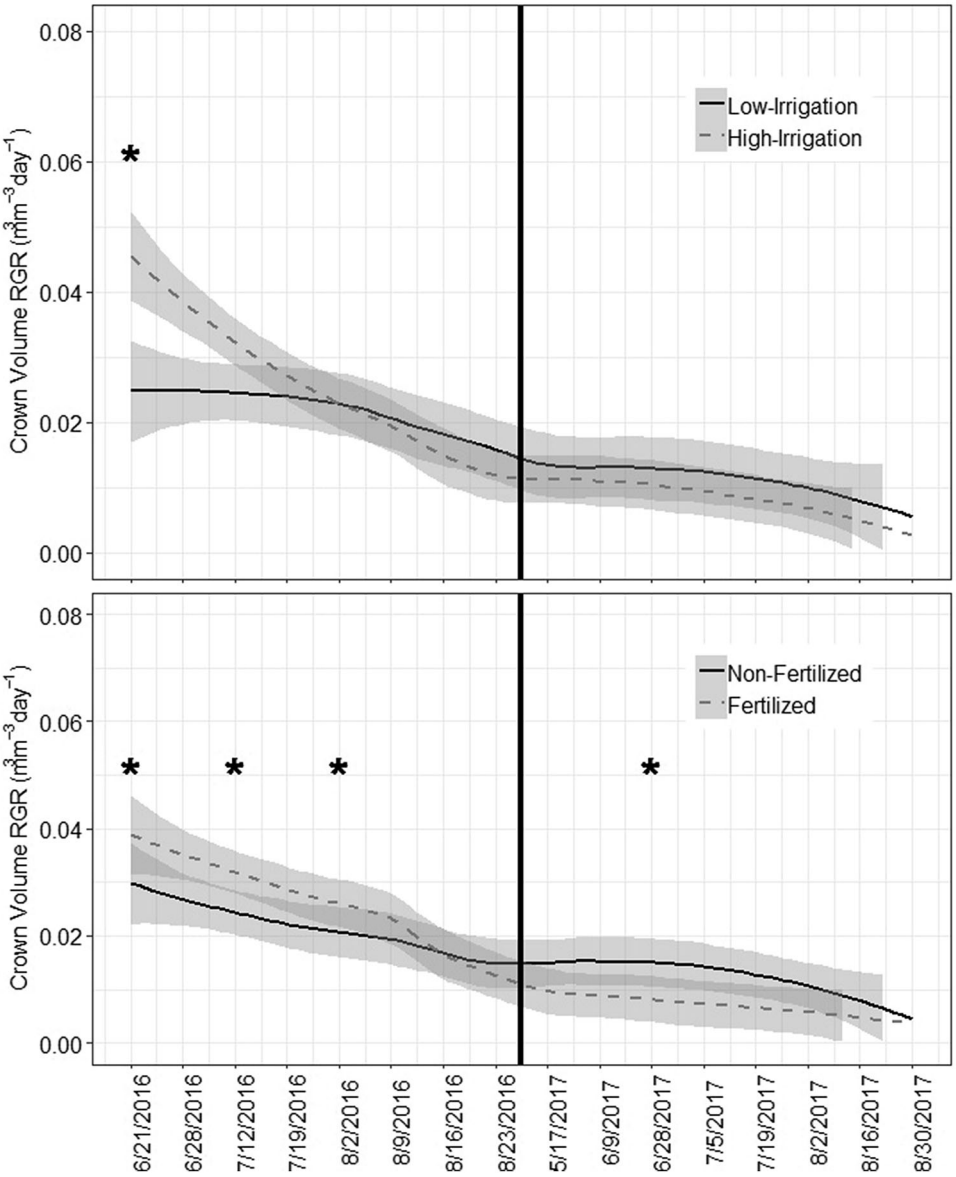

Fig. 2 Crown volume relative growth rates (RGR). The vertical bar separates growing seasons. Asterisks denote significant differences

It is likely that all plants were limited by resource availability, even in the fertilized-and-irrigated treatment, which reflects the nutrient-poor, sandy medium we deliberately used in this study. It is notable that total biomass per unit of total biomass N content (N-use efficiency; estimate: 73.7 ± 3.0 g; $p < 0.001$; R^2: 0.97) and total biomass per unit of total biomass P content (P-use efficiency; estimate: 103.9 ± 8.6; $p < 0.001$; R^2: 0.92) were linear and positive. In general, diminishing returns on N-use efficiency in crop yield studies are well-documented (Bodirsky and Müller 2014), but we had yet to reach these levels in this study given as these strong linear relationships had yet to reach a saturation point. This also suggests that greater N-accumulation and growth could be achieved with the addition of other nutrients. Nutrient accumulation may also be related to resilience to adverse conditions. The build-up of N and P in the plant tissues and its retention—as evidenced by little N being found in the soil—may enable Scotch broom to quickly respond to improved growing conditions in natural settings.

Conclusion

This study elucidated aspects of the ecology of a widespread, N-fixing invasive: Scotch broom. The findings provide estimates of resource-mediated N-accumulation in Scotch broom and total N inputs to a system after the plant dies or is chemically controlled. We expected to find evidence that greater N concentrations in the plant tissue were associated with greater growth and C-assimilation but found the opposite, indicating that a growth response from

Table 3 Estimated regression coefficients (standard errors in parentheses) and associated p-values from analyses of within-plant N and P on mean function and growth metrics of Scotch broom. Coefficient estimates and standard errors of log-transformed growth rate and biomass variables were back-transformed to show percent changes. Adjusted R^2 values are reported

	Aboveground biomass			Belowground biomass		
	β_1	p-value	R^2	β_1	p-value	R^2
Nitrogen (%)						
Physiology						
Assimilation ($\mu molCO_2\ m^{-2}\ s^{-1}$)	1.6 (2.6)	0.5	0.09	**− 2.0 (1.1)**	**0.09**	**0.08**
Transpiration (mmol $H_2O\ m^{-2}\ s^{-1}$)	**1.3 (0.8)**	**0.1**	**0.21**	− 0.5 (0.3)	0.13	0.21
Water-use efficiency ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$ mmol $H_2O\ m^{-2}\ s^{-1}$)	− 0.1 (0.2)	0.4	0.28	− 0.05 (0.7)	0.5	0.37
Bi-weekly soil water depletion ($m^3\ m^{-3}$)	− 0.06 (0.1)	0.3	0.09	0.05 (0.03)	0.12	0.17
Growth rate						
ln Basal diameter (mm $year^{-1}$)	**− 66.7 (82.2)**	**0.08**	**0.24**	22.1 (35.0)	0.5	0.13
ln Geometric mean crown width (cm $year^{-1}$)	− 55.1 (82.2)	0.2	0.06	22.1 (35.0)	0.5	0.13
ln Height (cm $year^{-1}$)	**− 59.3 (22.1)**	**0.1**	**0.22**	− 18.1 (35.0)	0.5	0.14
ln Crown volume ($m^3\ year^{-1}$)	− 87.8 (348.2)	0.2	0.13	− 50.3 (122.6)	0.4	0.07
Biomass						
ln Aboveground (g)	**− 90.0 (305.5)**	**0.1**	**0.23**	− 3.0 (101.4)	0.96	0.10
ln Belowground (g)	− 61.3 (171.8)	0.4	0.10	73.3 (64.9)	0.24	0.14
ln Total (g)	− 77.7 (267.0)	0.3	0.16	8.3 (82.2)	0.90	0.06
Phosphorus (mg kg^{-1})						
Physiology						
Assimilation ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	0.001 (0.001)	0.93	0.11	− 0.001 (0.002)	0.5	0.09
Transpiration (mmol $H_2O\ m^{-2}\ s^{-1}$)	0.00002 (0.0003)	0.96	0.07	− 0.001 (0.001)	0.4	0.12
Water-use efficiency ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$ mmol $H_2O\ m^{-2}\ s^{-1}$)	− 0.00003 (0.0001)	0.71	0.26	− 0.00002 (0.0002)	0.9	0.35
Bi-weekly soil water depletion ($m^3\ m^{-3}$)	− 0.00002 (0.00003)	0.57	0.05	− 0.00004 (0.00004)	0.7	0.01
Growth rate						
ln basal diameter (mm $year^{-1}$)	**− 0.1 (0.02)**	**0.004**	**0.45**	0.1 (0.7)	0.4	0.14
ln Geometric mean crown width (cm $year^{-1}$)	**− 0.1 (0.02)**	**0.04**	**0.21**	0.1 (0.06)	0.4	0.14
ln Height (cm $year^{-1}$)	**− 0.04 (0.02)**	**0.1**	**0.24**	− 0.1(0.07)	0.4	0.14
ln Crown volume ($m^3\ year^{-1}$)	**− 0.1 (0.06)**	**0.04**	**0.24**	− 0.2 (0.2)	0.2	0.15
Biomass						
ln Aboveground (g)	**− 0.1 (0.05)**	**0.01**	**0.39**	0.1 (0.2)	0.72	0.11
ln Belowground (g)	**− 0.1 (0.04)**	**0.03**	**0.32**	0.1 (0.1)	0.33	0.11
ln Total (g)	**− 0.1 (0.05)**	**0.03**	**0.35**	0.1 (395.3)	0.66	0.06

Significant values are in bold

N is dependent on the availability of other resources. This indicates that the role of BNF in Scotch broom's competitive advantage over native species is likely site-dependent.

Acknowledgements We would like to thank James Dollins, Alyssa Peter, LeRoy Turner and Dave Peter for their help setting up and maintaining this study, as well as taking field measurements. Funding for this project was provided by the USDA National Institute for Food and Agriculture (Grants.Gov Number: GRANT11325729).

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