Functional Traits Exert More Control on Root Carbon Exudation than Do Short-Term Light and Nitrogen Availability in Four Herbaceous Plant Species

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Eileen Thorsos

University Program in Ecology
Duke University

Date:_____
Approved:

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Robert Jackson, Co-Supervisor

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Justin Wright, Co-Supervisor

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Chantal Reid

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Daniel Richter

Thesis submitted in partial fulfillment of
the requirements for the degree of Master of Science in the University Program
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ABSTRACT

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Abstract

Root carbon exudation is a critical process in the soil carbon cycle, and how both environmental conditions and plant traits influence exudation remains uncertain. I studied relationships between environmental conditions, plant traits, and carbon exudation in four herbaceous plant species: *Asclepias incarnata*, *Microstegium vimineum*, *Panicum virgatum*, and *Scirpus cyperinus*. Mature individuals were given short-term factorial treatments of light and N, and exudates were collected over 8 hours in carbon-free hydroponic nutrient solution. I measured size traits (biomass, leaf area, root length, and root volume), photosynthetic rate (leaf-level and whole-plant), and tissue N concentrations (root, stem, and leaf percent N and C:N ratios). Neither light nor N treatments affected exudation, while exudation varied with species and plant traits. Species alone substantially explained mass-specific exudation (estimated $R^2 = 0.38$). Size strongly predicted both total and mass-specific exudation, interacting with species (estimated $R^2 = 0.52$ and 0.48, respectively). Generally, larger individuals exuded more overall but less per unit mass, although larger *M. vimineum* plants exuded more per unit mass. Whole-plant photosynthetic rate was weakly related to total exudation (estimated $R^2 = 0.17$), and tissue N concentration moderately predicted mass-specific exudation (estimated $R^2 = 0.23$). Other researchers have found that high light and low nitrogen availability stimulate exudation; my results indicate that this relationship is not straightforward. Plant traits, however, significantly explained variation in exudation, including some variation across species, supporting trait-based analyses of plant species' effects on ecosystem processes.

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1.1 Introduction

Carbon compounds exuded from plant roots play important roles in soil carbon and nutrient cycling (e.g., Dakora and Phillips 2002, van Hees et al. 2005, Hamilton et al. 2008, Hogberg et al. 2008, Phillips et al. 2011). However, while we understand some of the fundamental drivers of carbon exudation, exudation remains perhaps the most uncertain component of the terrestrial carbon cycle (Hungate et al. 1997, Jones et al. 2004). Both environmental conditions and characteristics of the plant act and interact to influence rates of carbon exudation, and how these factors affect root exudation is a critical question in carbon-cycle research. In this study, I asked three questions: *1. How do light and nitrogen availability influence carbon exudation rates? 2. How do plant traits such as size, photosynthetic rate, and tissue nitrogen influence carbon exudation? 3. To what extent does variation in these traits across species explain species' variation in exudation rates?*

A plant's environment influences both the availability of carbon for exudation and demand by the plant for the benefits derived from exudation. When shading inhibits photosynthesis, exudation can swiftly decrease, suggesting that internal carbon supply is an important control on exudation rates (Rovira 1969, Dilkes et al. 2004, Hill et al. 2007). Factors such as nitrogen limitation can induce plants to elevate carbon exudation rates: Labile exudates can provide easily-accessed organic carbon to soil microbes, likely stimulating a food web that decomposes recalcitrant soil organic matter and induces organic nitrogen to mineralize, making it more available to plants (called the 'priming effect'; Kuzyakov 2002, Jones et al. 2004, Fransson and Johansson 2010, Kuzyakov 2010). Plants with greater carbon supply may also be better equipped to actively release exudates into the soil in response to low nitrogen supply and other environmental stressors (Phillips et al. 2009, Phillips et al. 2011). However, interactions between the

effects of nutrient and carbon supply on root carbon exudation rates remain poorly explored.

Exudation can also be controlled by variation in plant traits within and across species. Such traits may both respond to environmental conditions—e.g., changes in photosynthesis due to light availability—and have independent controls—e.g., species' physiological constraints on photosynthesis. Variation in root architectural traits such as root surface area, branching patterns, tip density, or overall root system size can drive passive levels of exudation (McDougal and Rovira 1970, Jones et al. 2004, Badri and Vivanco 2009). Further, when plants actively elevate exudation above baseline, e.g., in response to environmental stimuli, the degree of this elevation may be shaped by additional plant traits. For example, photosynthetic activity may constrain carbohydrate supply and thus the plant's capacity to release exudates on demand. Additionally, nitrogen limitation should result in low tissue nitrogen concentrations, and such nitrogen-limited plants should elevate exudation in order to stimulate turnover of organic soil nitrogen. However, we do not yet have a strong understanding of the relationships between root carbon exudation and either photosynthetic input or tissue nitrogen concentration, and overall we lack a trait-based understanding of plant-level controls on exudation within and across species.

I investigated several hypotheses about links between root carbon exudation, plant traits, and light and nitrogen availability for four herbaceous plant species:

Hypothesis 1: Light-Nitrogen Interaction. Nitrogen limitation elevates exudation rates, and the degree of this elevation is controlled by carbon fixation: High light allows greater carbon fixation and thus greater exudation in response to low nitrogen.

Hypothesis 2: Traits. (A.) *Size*. Larger individuals exude more. (B) *Photosynthesis.* Individuals with higher photosynthetic input have higher exudation rates. (C) *Tissue Nitrogen Status.* Individuals with low tissue nitrogen concentration due to nitrogen limitation elevate exudation in low nitrogen conditions.

Hypothesis 3: Species Differences. Variation in exudation across species is correlated with variation in key traits, including size, photosynthetic rate, and nitrogen status.

1.2 Materials and Methods

1.2.1 Growth Conditions

I selected four study species with widespread distribution in the southeastern United States. From a pilot study (data not shown), I identified two species with variable rates of exudation (*Asclepias incarnata*, swamp milkweed, and *Panicum virgatum*, switchgrass) and one species with consistently low exudation (*Scirpus cyperinus*, woolgrass). All three species are native in much of the eastern United States, and *Panicum virgatum* is a potentially important biofuel crop. I also included *Microstegium vimineum*, Japanese stiltgrass, an invasive graminoid common in moist areas of the southeastern United States. To my knowledge, there are no published accounts of carbon exudation in these species, with minimal assessments in the *Panicum* and *Scirpus* genera (ISI Web of Knowledge search, December 9, 2011; Vancura and Garcia 1969, Dias-Arieira et al. 2003, Kim and Kang 2008, Mucha et al. 2008, 2010).

Seeds of all four species were germinated in May 2010. *M. vimineum* seeds were started approximately 2 weeks after the others. The other three species were treated with 0.14 mM gibberellic acid for 3 days to speed germination. *P. virgatum*, *A. incarnata*, and *M. vimineum* all germinated and grew rapidly, while *S. cyperinus* grew more slowly.

In mid-June, approximately 6 weeks (4 weeks for *M. vimineum*) after seeds were planted, 20 individuals of each species were potted in 3:1 gravel-vermiculite mix in 4-L pots. This potting medium was chosen to support growth and allow later transplanting with minimal root damage. Plants were randomly assigned to carts, with one individual of each species per cart. Carts were arranged in five blocks of four (20 individuals of each species). Because of several damaged individuals, the central block was later used to replace several damaged individuals, resulting in sixty-four study individuals arranged in four blocks of 16 plants.

Plants were watered twice daily and fertilized 3 times per week with 75 ppm (15 ppm N) Peter's Professional Peat-Lite Special nutrient solution. They grew in an open-top greenhouse maintaining ambient North Carolina temperature (mean 29°C, with maximum 40°C and minimum 21°C during the growth period) and relative humidity (mean 72%), but excluding natural rainfall.

In late July 2010, all plants were transferred to a hydroponic medium to allow exudate collection in liquid solution. All plants were un-potted and their roots rinsed. Each plant was returned to an individual pot lined with a fresh 92 oz. sterile Whirl-Pak bag and placed into hydroponic solution (1/4 strength Hoagland's solution), constantly aerated with an individual bubbler. The pot surface was covered with black plastic to minimize light entry to the pot. Nutrient solution was refilled daily and exchanged weekly. Plants were allowed to adjust to the hydroponic rooting medium for a minimum of 20 days before beginning nutrient and light treatments. As plants matured, they were supplied with 3/8 strength Hoagland's solution to prevent micronutrient limitation (except for the first block, which was harvested previously).

No pest presence was observed until near the end of the experiment, and one pesticide application of Safari SC was applied. All nutrient solutions were replaced the following day. When exudates were collected, all *A. incarnata* and all but one *P. virgatum* individuals had flower buds or were flowering; only three *S. cyperinus* individuals and no *M. vimineum* individuals had reached flowering.

1.2.2 Treatments

I studied sixty-four plants in four replicate blocks of a factorial treatment array of 4 species x 2 light levels x 2 nitrogen levels (HLHN = high light, high nitrogen; HLLN = high light, low nitrogen; LLHN = low light, high nitrogen; and LLLN = low light, low nitrogen). The durations of light and nitrogen treatments were designed to elicit stress without influencing biomass or root:shoot bulk carbon allocation and were based on an assessment of literature and on a small pilot study with these four species (pilot data not shown; Best 1980, Lambers and Posthumus 1980, Jones et al. 1981, Hole and Dearman 1993, Macduff et al. 1993). "High" treatments maintained baseline conditions: ambient light and full N supply in the Hoagland's nutrient solution. Low light was applied with shade structures for one full day prior to exudation collection (PVC frame structure, with black shade cloth layered three times to produce 90% to 95% light decrease). Low nitrogen treatments were applied as 1/10 full N for 5 days prior to exudate collection. All nutrient solutions were brought to pH 6.0 with NaOH additions prior to use. All treatments were sustained through the exudate collection period.

1.2.3 Exudate Collection and Measurement

The day of exudation collection, all individuals in a block were removed from their nutrient solution and light treatments, and their roots were rinsed three times with DDI H_2O. Plants were handled as gently as possible to minimize damage. They were

returned to 3 L of nutrient solution free of dissolved organic carbon (DOC) at the treatment N concentration, with a fresh WhirlPak, bubbler, air tube, and black cover. Nutrient solution was made DOC-free by omitting Fe, which is normally chelated with EDTA, an organic compound, to remain in solution. A similar blank (no plant) was established for each treatment within the block.

For exudate collection, I pumped a syringe three times to mix the nutrient solution before drawing samples. All exudate samples were filtered with a 0.22-micron Millex GV filter (Millipore). An initial sample of nutrient solution was taken immediately upon placing the plant in the C-free nutrient solution (30 mL), and shade treatments were re-applied. Subsequent samples (120 mL) were collected at 4 and 8 hours of incubation. At each sampling, the plant, pot, and nutrient solution were weighed to estimate the liquid volume at each time point due to changes from transpiration losses. Samples were immediately chilled on ice and frozen at the end of the sampling period. Samples remained frozen for 4-5 weeks until analysis on a Shimadzu TOC analyzer for total organic carbon content.

1.2.4 Trait Measurements

Leaf, shoot, and root traits were measured on all plants. During the exudates collection period, leaf-level photosynthetic rates were measured with a LICOR 6400. To standardize measurements, photosynthetically active radiation (PAR) in the LICOR cuvette was set to 1000 μmol quanta m^{-2} s^{-1} for high light treatments and 50 μmol quanta m^{-2} s^{-1} for low light treatments. I measured leaf photosynthetic rate on three leaves per individual and averaged the result. The following day, all plants in the measured block were harvested. Leaf area was measured with a table-top rolling-belt leaf area meter (LICOR 3100), and shoot samples (leaf and stem) were dried at 60ºC and weighed. For

all species but *M. vimineum*, I measured total leaf area. Due to its high leaf area, for *M. vimineum* I harvested a substantial subsample (minimum 40% of total shoot mass) and determined specific leaf area (SLA) from leaf area and leaf mass. Then, I estimated total *M. vimineum* leaf area from SLA, shoot:stem ratio, and total shoot mass. Rates of potential whole-plant photosynthesis were estimated by multiplying the leaf photosynthetic rate by total leaf area.

Root traits were measured on subsamples and total root systems, depending on the trait. Representative subsamples of fresh roots were stained with toluidene blue and scanned in black and white using an Epson Expression 10000XL scanner. Images were analyzed for subsample root length, root area, and root volume using WinRHIZO (Regular v. 2005c). All root samples (bulk and subsample) were dried at 60ºC and weighed. Total root length, area, and volume were estimated by mass from the subsample measurements. One *S. cyperinus* individual was excluded from all analyses due to lost root mass. Root:shoot ratio was calculated from total belowground and total aboveground biomass. Leaf, stem, and root samples were ground with a Wiley Mill (augmented with mortar and pestle where needed) and analyzed for percent C and N on a Shimadzu CHN Analyzer.

1.2.5 Statistical Analyses

Carbon exudation rates, estimated for the first and second four-hour periods separately, were calculated as

$$exudation = \frac{[C]_1 V_1 - [C]_0 V_0}{t_1 - t_0} - b \,, \qquad \text{Eq. 1}$$

where [C] is the carbon concentration, V is the liquid volume, t is time (0 = initial, 1 = final), and b indicates the mean rate of change across all blanks. The rate of change in

blanks did not vary statistically with block or treatment yet varied substantially overall, so changes in all blanks were averaged and subtracted from each calculated carbon exudation rate. For each individual, I used the higher rate from the two time periods as the total (whole-plant) exudation rate, and I divided total exudation by root mass to calculate root-mass-specific exudation rates.

I tested my hypotheses involving the relationships between treatments, traits, species, and carbon exudation using a series of linear mixed effects models (the lme function in R, package nlme), with total and root mass-specific exudation as the response variables (Table 1). Because of the number of models I built, I set α to 0.01 to minimize Type I error. To test hypotheses involving four or more traits (nitrogen-related traits and size-related traits), many of which were collinear, I first built separate principal components analyses (PCAs) from the target traits relevant for each hypothesis (Table 1). For each individual, I included in my models the PCA loading scores of the first two components. (In all cases, the third component detracted from statistical power and did not substantially enhance the fit of the PCA to the data.)

For total and mass-specific exudation separately, I compared the relative strength of the mixed effects models using Akaike Information Criterion (AIC) scores and Akaike weights to determine which models had the greatest analytical support. Lower AIC scores indicate greater relative model support, and differences greater than 2 points indicate substantial differences in model support. For both measures of exudation I determined the Akaike weight (w) of each model, which is given by

$$w_i = \frac{\exp(-\frac{1}{2}(AIC_i - AIC_{\min})}{\sum_{r=1}^{R} \exp(-\frac{1}{2}(AIC_r - AIC_{\min})} \quad , \qquad \text{Eq. 2}$$

Table 1: Hypotheses, relevant traits, and specific models built to test the hypotheses. Terms separated by 'x' were included as all individual terms as well as all possible interactions. Model 1 includes species, treatment, and block effects only, with no traits. In these models, L = light treatment, N = nitrogen treatment, Sp = species, B = block, Photo.L = leaf photosynthetic rate, Photo.W = whole-plant photosynthetic rate, PC1.S and PC2.S = principal components of size traits, and PC1.N and PC2.N = principal components of nitrogen traits.

Hypothesis	Model	Traits	Model
1. Light and Nitrogen	*1. Light & Nitrogen*	N/A	Y = L x N x Sp + B
2. Traits	*2. Size*	Shoot mass, leaf area, root mass, root length, root area, root volume	Y = (PC1.S + PC2.S) x Sp + B
	3. Leaf Photosynthesis	Leaf photosynthetic rate	Y = Photo.L x Sp + B
	4. Whole-Plant Photosynthesis	Whole-plant photosynthetic rate	Y = Photo.W x Sp + B
	5. Tissue Nitrogen	Leaf, stem, and root % N and C:N ratio	Y = (PC1.N + PC2.N) x Sp + B
3. Species Differences	*6. Species Only*	N/A	Y = Sp + B

where the Akaike weight (w_i) indicates the likelihood that the *i*th model is the best fit model out of the total set of *R* models, AIC_i indicates the *i*th AIC value, and AIC_{min} indicates the lowest AIC value out of the set of *R* models. Akaike weights range from 0 to 1, and the models with the strongest weights were determined.

Linear mixed effects models have no consensus protocol to determine absolute measures of goodness-of-fit, such as an R^2 value, and proposed measures have unclear interpretations (Vonesh et al. 1996, Edwards et al. 2008, Liu et al. 2008). To obtain adjusted R^2 values that roughly estimate the amount of variation in exudation explained by each linear mixed effects model, I also built fixed effects linear regressions with the same input terms but with block as a fixed effect. I confirmed that the mixed and the fixed effects models detected generally similar relationships among the variables; further, in t-tests none of the residuals for the two models were significantly different. However, because mixed effects models and fixed effects models are parameterized differently, these R^2 values are rough estimates of the variation explained by the mixed effects models that I report.

For Hypothesis 1, that exudation was controlled by the interaction of light and nitrogen availability, I first built a basic linear mixed effects model to test for effects of light and nitrogen on carbon exudation rates, allowing for interactions, controlling for species, and with block as a random effect (Model 1, Table 1). To enhance statistical power, in all subsequent models for all hypotheses I removed the treatment components that did not have a significant or marginally significant relationship with carbon exudation in this initial model. For Hypothesis 2, I built four mixed effects models (Models 2 to 5): size (based on a PCA of shoot biomass, leaf area, root biomass, root length, root surface area, and root volume), leaf photosynthesis, whole-plant

photosynthesis, and nitrogen (based on a PCA of percent N and C/N for leaves, stems, and roots). In each of these models, I included a species term and allowed interactions between traits and species.

To test Hypothesis 3, that traits explain variation in exudation across species, I compared the traits-plus-species models above to a species-only model and to traits-only models. I assessed how species and traits each contributed to the explanatory power of the traits-plus-species models. If the estimated R^2 of the combined model was less than additive from the species-only and traits-only estimated R^2 values, it implied that species and traits explained similar variation. Remaining variation explained by species – or interactions between a trait and species – indicated contributions of traits not included in this analysis.

1.3 Results

1.3.1 Trait Measurements

Most measured traits varied with species, and treatment influenced both photosynthesis and tissue nitrogen concentration. *M. vimineum* individuals averaged the largest shoot mass (stem and leaf combined), followed by *A. incarnata*, *P. virgatum*, and *S. cyperinus* ($p = 0.001$, Table 2). Although *A. incarnata* individuals averaged the largest root mass, followed in order by *M. vimineum*, *P. virgatum*, and *S. cyperinus*, only *S. cyperinus* root mass was significantly lower than that of the other species ($p = 0.001$, Dunnett's modified Tukey-Kramer pairwise multiple comparison test, Table 3). Neither total biomass nor root-to-shoot ratio were significantly altered by the short-term light or nitrogen treatment ($p > 0.10$ in both cases). Low light treatments reduced photosynthesis by 90.0% on average, with significant differences in photosynthesis by species, light treatment, and their interaction ($p \leq 0.0001$, Figure 1). Species also differed

Table 2: Shoot traits averaged by species and treatment (n = 4 except *, where n = 3). See Figures 1 and 2 for leaf photosynthetic rates, leaf N (%), and stem N (%). Standard errors are given in parentheses.

Species	Treatment	Shoot Mass (g)	Root: Shoot ratio	Total Leaf Area (cm^2)	Whole-plant Photosynthetic Rate ($\mu mol\ CO_2\ s^{-1}$ per plant)	Leaf C/N	Stem C/N
A. incarnata	HL HN	30.2 (7.6)	0.16 (0.03)	5,096 (1365)	9.06 (2.95)	7.6 (0.2)	43.9 (7.8)
	HL LN	31.6 (7.1)	0.13 (0.01)	4,981 (1049)	6.60 (2.33)	9.2 (0.8)	50.7 (2.6)
	LL HN	28.3 (6.4)	0.11 (0.02)	4,745 (1094)	0.90 (0.27)	7.8 (0.3)	31.8 (4.5)
	LL LN	24.5 (6.1)	0.14 (0.004)	4,055 (1120)	0.83 (0.39)	8.2 (0.2)	37.1 (4.7)
M. vimineum	HL HN	41.1 (5.2)	0.06 (0.01)	9,420 (1227)	17.58 (3.82)	12.5 (0.1)	22.4 (1.4)
	HL LN	42.4 (7.9)	0.08 (0.01)	7,674 (2360)	14.11 (4.94)	12.6 (0.4)	26.2 (1.9)
	LL HN	43.5 (10.1)	0.07 (0.01)	10,895 (1827)	2.88 (0.84)	11.6 (0.2)	21.7 (1.3)
	LL LN	45.9 (8.5)	0.06 (0.01)	10,492 (2195)	2.77 (1.13)	13.4 (0.4)	27.6 (1.0)
P. virgatum	HL HN	14.3 (4.9)	0.12 (0.01)	1,663 (556)	4.12 (1.94)	16.2 (2.2)	21.3 (3.2)
	HL LN	21.7 (8.6)	0.16 (0.02)	2,194 (690)	4.76 (1.69)	15.0 (0.7)	25.6 (1.8)
	LL HN	12.5 (4.4)	0.11 (0.02)	1,763 (695)	0.17 (0.10)	13.0 (0.5)	19.5 (1.4)
	LL LN	9.5 (1.9)	0.14 (0.03)	1,093 (247)	0.19 (0.08)	13.5 (0.2)	20.4 (0.7)
S. cyperinus	HL HN*	2.7 (0.8)	0.12 (0.04)	457 (134)	0.56 (0.29)	13.7 (0.2)	18.2 (0.12)
	HL LN	2.9 (1.2)	0.08 (0.01)	483 (238)	0.53 (0.36)	14.3 (0.5)	21.3 (2.7)
	LL HN	2.0 (0.6)	0.10 (0.01)	306 (74)	0.03 (0.01)	12.9 (0.2)	15.8 (0.9)
	LL LN	2.7 (0.7)	0.12 (0.01)	473 (102)	0.05 (0.05)	13.6 (0.4)	20.2 (1.7)

Table 3: Total exudation, mass-specific exudation, and root traits, averaged by species and treatment (n = 4 except *, where n = 3). See Figure 2 for root N (%). Standard errors are given in parentheses.

Species	Treatment	Total exudation (mg C h^{-1} per plant)	Mass-specific exudation (mg C h^{-1} g^{-1})	Root mass (g)	Total root length (m)	Total root area (cm^2)	Total root volume (cm^3)	Root C/N
A. incarnata	HL HN	0.21 (0.09)	0.05 (0.02)	4.2 (0.8)	54.8 (12.5)	6,547 (1258)	63.2 (10.2)	9.3 (0.6)
	HL LN	0.34 (0.18)	0.07 (0.07)	4.4 (1.3)	46.1 (10.3)	5,766 (1524)	58.1 (17.6)	12.6 (0.5)
	LL HN	0.22 (0.11)	0.08 (0.06)	2.9 (0.3)	34.6 (8.9)	4,162 (870)	40.1 (6.5)	9.6 (0.8)
	LL LN	0.14 (0.07)	0.06 (0.06)	3.3 (0.9)	43.4 (9.5)	4,873 (1020)	43.6 (8.7)	10.5 (0.8)
M. vimineum	HL HN	0.59 (0.22)	0.18 (0.19)	2.6 (0.5)	69.1 (13.3)	4,994 (1061)	28.9 (6.8)	17.9 (1.3)
	HL LN	0.40 (0.18)	0.11 (0.10)	3.3 (0.5)	86.7 (7.7)	5,737 (611)	30.5 (3.9)	20.1 (1.7)
	LL HN	0.32 (0.16)	0.09 (0.13)	2.9 (0.5)	57.3 (18.2)	5,375 (1316)	34.4 (7.5)	19.6 (3.3)
	LL LN	0.17 (0.12)	0.07 (0.10)	2.6 (0.5)	72.9 (10.8)	4,759 (763)	25.5 (4.9)	18.8 (2.8)
P. virgatum	HL HN	0.24 (0.09)	0.18 (0.10)	1.7 (0.5)	25.0 (8.2)	1,611 (508)	8.3 (2.5)	16.7 (1.5)
	HL LN	0.19 (0.10)	0.11 (0.13)	3.5 (1.2)	37.2 (11.5)	2,789 (895)	16.7 (5.6)	17.7 (1.4)
	LL HN	0.22 (0.10)	0.37 (0.43)	1.6 (0.6)	25.5 (10.5)	1,612 (648)	8.2 (3.3)	14.2 (1.7)
	LL LN	0.14 (0.07)	0.16 (0.15)	1.2 (0.4)	14.6 (3.5)	1,047 (227)	6.1 (1.3)	15.8 (1.6)
S. cyperinus	HL HN*	0.10 (0.05)	0.38 (0.10)	0.05 (0.02)	6.7 (1.6)	451 (85)	2.4 (0.4)	22.6 (1.0)
	HL LN	0.08 (0.07)	0.37 (0.29)	0.07 (0.12)	6.3 (3.1)	410 (200)	2.1 (1.0)	24.2 (2.1)
	LL HN	0.07 (0.06)	0.35 (0.20)	0.06 (0.06)	4.7 (1.2)	298 (76)	1.5 (0.4)	22.6 (1.6)
	LL LN	0.13 (0.07)	0.38 (0.14)	0.07 (0.11)	6.3 (1.5)	453 (106)	2.6 (0.6)	23.3 (1.0)

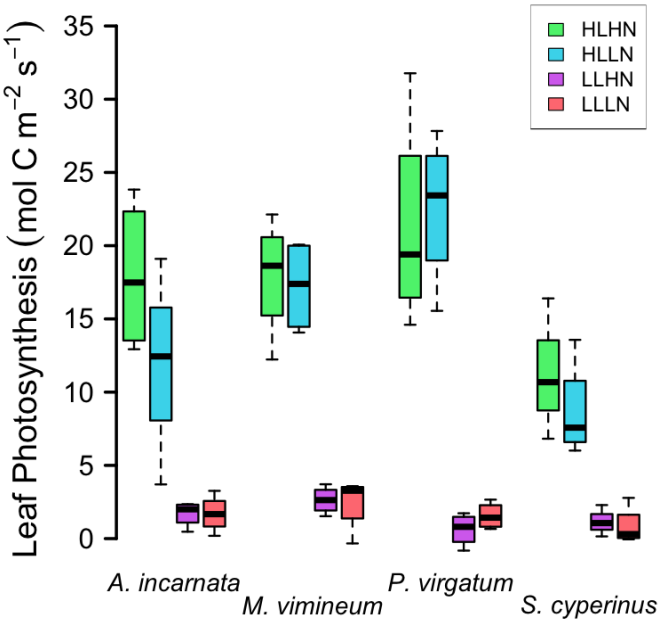

Figure 1: Leaf-level photosynthetic rates (μmol CO_2 m^{-2} s^{-1}) by species and treatment, where HLHN = high light, high nitrogen, HLLN = high light, low nitrogen, LLHN = low light, high nitrogen, and LLLN = low light, low nitrogen (n = 4 in all cases except S. cyperinus HLHN, where n = 3). Black lines indicate the median, box hinges indicate the first and third quartiles, and whiskers indicate the full range of the data. Shade treatments significantly reduced photosynthetic rates ($p \leq 0.0001$) by 90% on average, with some variation by species.

in percent N in roots and shoots ($p < 0.0001$), and both N and light treatments affected tissue percent N above- and belowground, with higher tissue N concentrations in the high N treatments (Figure 2).

1.3.2 Environment and Exudation

Total maximum exudation varied from -0.1 mg C/h to 1.63 mg C/h, and root mass-specific exudation varied from -0.06 mg C/h/g root to 1.00 mg C/h/g root (Figure 3, Table 3). Counter to Hypothesis 1, there were no significant effects of light or nitrogen treatments alone or in interaction for either measure of exudation (Table 4, Figure 3). In Model 1, total exudation varied marginally by species, with the highest rates by *M.*

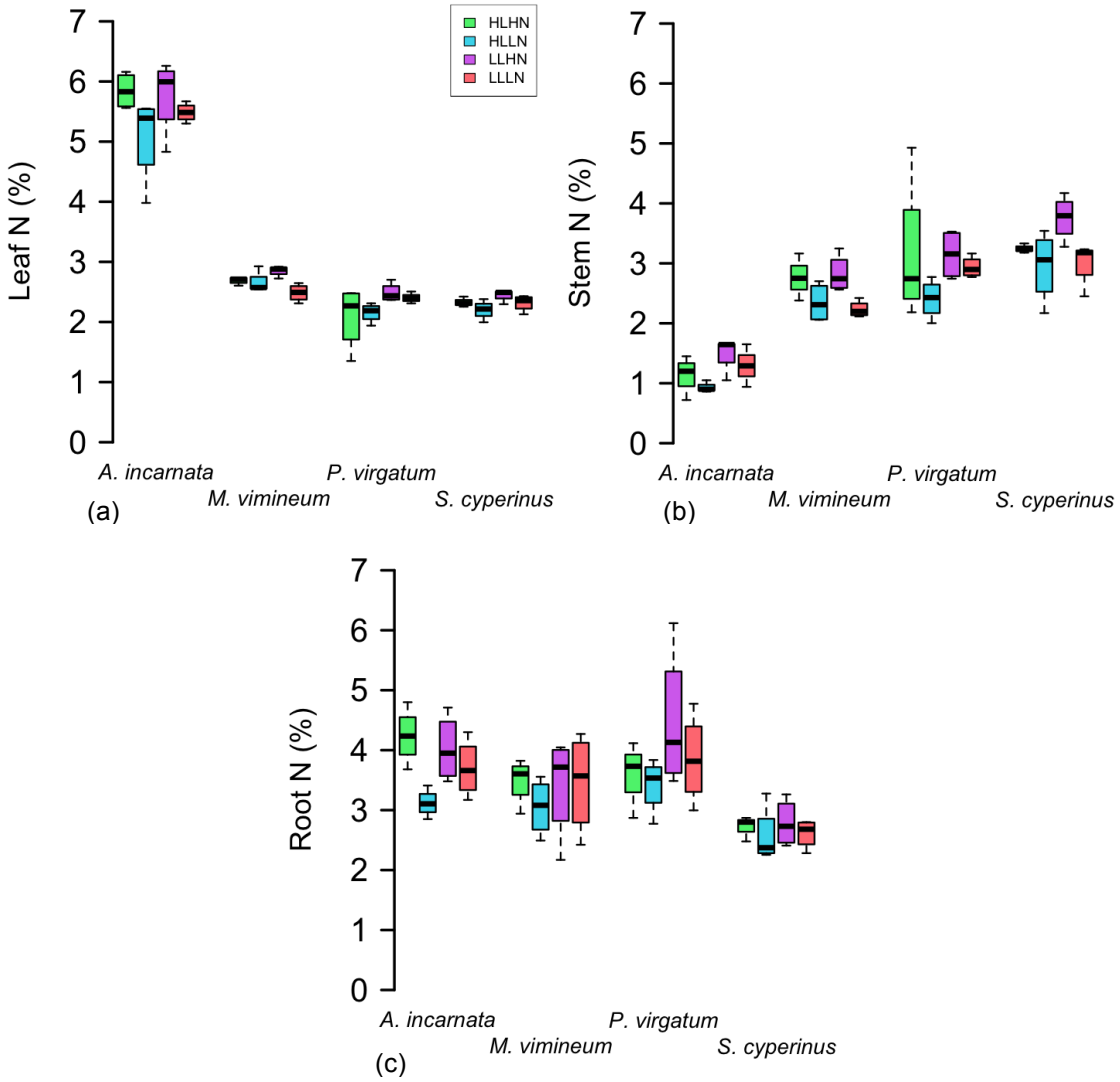

Figure 2: Tissue N (%) varied with nitrogen and light treatments for all tissue types: (a) leaf, (b) stem, and (c) root. Low nitrogen treatments reduced tissue N concentration. In all cases n = 4, except for *S. cyperinus* HLHN, where n = 3. Black lines indicate the median, box hinges indicate the first and third quartiles, and whiskers indicate the full range of the data.

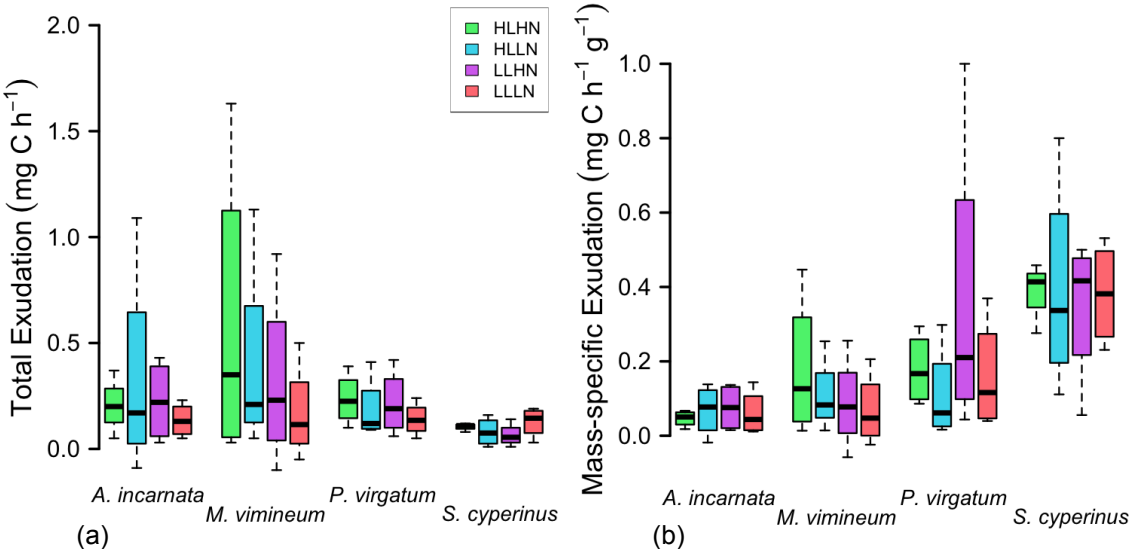

Figure 3: Boxplots of C exudation rates by species and treatment. Linear mixed effects models show no effect (main or interaction) of either light or nitrogen for (a) total exudation (mg C h^{-1}) or (b) mass-specific exudation (mg C h^{-1} g^{-1}). Species is a marginally significant predictor of total exudation ($p = 0.08$) and a significant predictor of mass-specific exudation ($p < 0.0001$). Negative exudation values indicate that the rate of exudation was less than the combined effects of respiration in solution and plant uptake of the initial carbon in solution. In all cases n = 4, except for *S. cyperinus* HLHN, where n = 3. Black lines indicate the median, box hinges indicate the first and third quartiles, and whiskers indicate the full range of the data.

vimineum and the lowest by *S. cyperinus* ($p = 0.08$; see Table 4 for all AIC scores). In all species but *S. cyperinus*, individuals in the low-light, low-nitrogen treatments had the lowest total exudation rates, but the species-by-treatment interaction was not a significant predictor of total exudation rate. Mass-specific exudation strongly varied by species in Model 1, with mass-specific exudation highest in *S. cyperinus* and lowest in *A. incarnata* ($p < 0.0001$, estimated $R^2 = 0.29$). Because light and nitrogen were not significant predictors in these models, I excluded these terms from subsequent models.

1.3.3 Traits and Exudation

Size strongly predicted exudation. Consistent with Hypothesis 2A, total exudation generally increased with plant size ($p<0.0001$, estimated $R^2 = 0.52$, Model 2),

Table 4: Models, AIC values, estimated R^2 values, Akaike weights, significant variables and their p-values, where ‡ ≤ 0.10, † ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, **** ≤ 0.0001. Values in parentheses indicate results from comparable models without a species term. Interaction terms are represented by the two individual terms separated by the symbol *.

Model	*Y = Total exudation*					*Y = Mass-specific exudation*				
	AIC	**Est. R^2**	**w_i**	**Significant terms**	**p-values**	**AIC**	**Est. R^2**	**w_i**	**Significant terms**	**p-values**
1. Light & Nitrogen										
Y = N x L x Sp + B	89.1	0.03	6.9 e-13	Species	‡	36.4	0.31	1.5 e-09	Species	****
2. Size Traits				PC1.S	****					
Y = (PC1.S + PC2.S) x Sp + B	33.1	0.52	0.99	PC2.S	‡	-4.2	0.48	0.99	PC1.S	****
		(0.26)		Species	‡		(0.31)		Species	†
				PC1.S*Species	**				PC1.S*Species	**
				PC2.S*Species	†					
3. Leaf Photosynthesis		0.12					0.37		Photo.L	‡
Y = Photo.L x Sp + B	76.5	(0.06)	3.8 e-10	Species	‡	15.4	(0.06)	5.5 e-05	Species	****
4. Whole-Plant Photosynthesis		0.12					0.39		Photo.W	†
Y = Photo.W x Sp + B	68.7	(0.17)	1.9 e-08	Photo.W	**	5.2	(0.09)	9.0 e-03	Species	****
5. Tissue Nitrogen		0.05					0.34		PC1.N	***
Y = (PC1.N + PC2.N) x Sp + B	76.4	(0.05)	4.0 e-10	Species	‡	18.2	(0.23)	1.5 e-05	Species	**
Species Only										
Y = Sp + B	40.8	0.14	–	Species	†	-24.1	0.38	–	Species	****
Size & Whole-Plant Photosynthesis										
Y= PC1.S + Photo.W + B	43.2	0.25	–	PC1.S	****	–	–	–	–	–
Size & Tissue N									PC1.S	****
Y= PC1.S + PC1.N + B	–	–	–	–	–	-19.4	0.34	–	PC1.N	‡

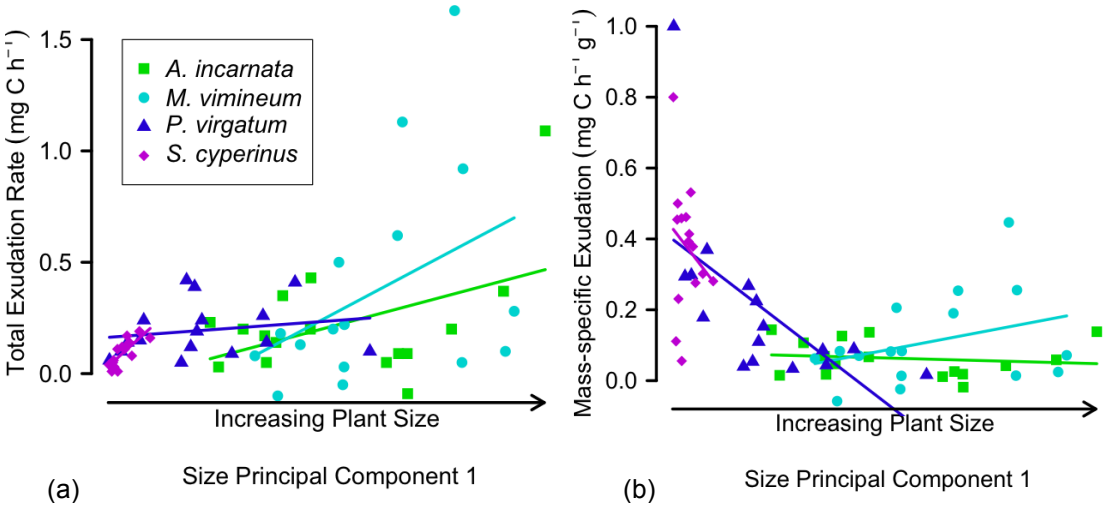

Figure 4: (a) Total exudation rate and (b) mass-specific exudation as a function of size and species. Size is represented as the first principal component of a PCA of shoot mass, leaf area, root mass, root length, and root volume. Size ($p < 0.0001$), species ($p < 0.05$), and their interaction ($p < 0.008$) are significant predictors of both measures of exudation.

with interactions between species and size traits (Figure 4a). For total exudation, the second size principal component was marginally significant, with a nearly significant interaction with species (Table 4). I observed little relationship in *P. virgatum* between total exudation and size; in *A. incarnata*, total exudation increased modestly with size; and in *M. vimineum* and *S. cyperinus*, exudation increased strongly with size. Mass-specific exudation generally decreased with plant size, but this relationship varied by species ($p < 0.0001$, estimated $R^2 = 0.48$, Model 2, Figure 4b). In *S. cyperinus* and *P. virgatum*, mass-specific exudation decreased as plant size increased, while *A. incarnata* appeared to have no relationship between mass-specific exudation and size and for *M. vimineum* mass-specific exudation increased with plant size.

Leaf photosynthesis did not significantly predict total exudation (Model 3, Table 4) and marginally predicted mass-specific exudation ($p = 0.07$). Whole-plant photosynthetic rate, which scales leaf-level photosynthetic rates by total leaf area

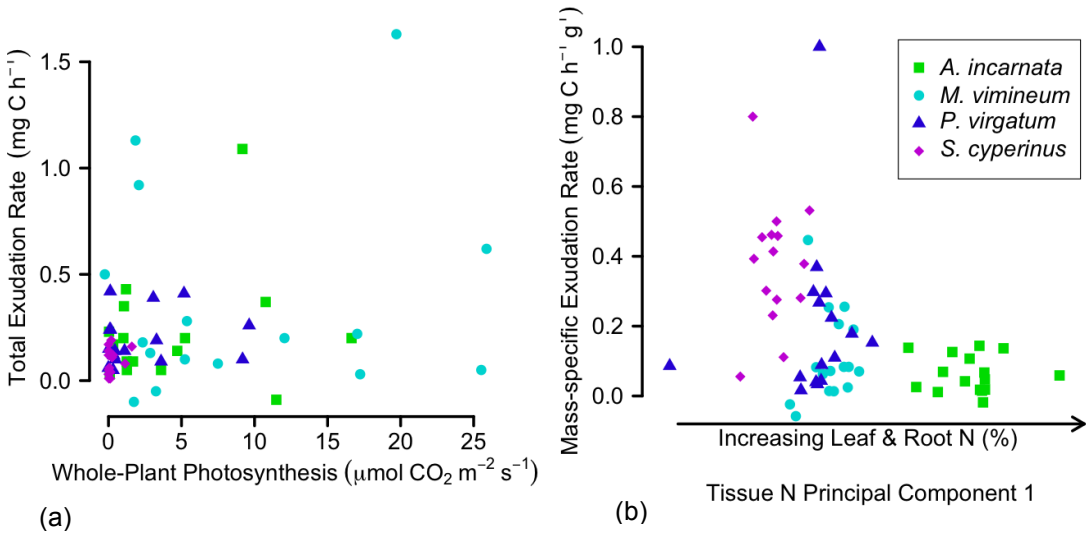

Figure 5: (a) Total exudation by species as a function of whole-plant photosynthetic rate and (b) mass-specific exudation by species as a function of tissue nitrogen traits (principal component 1 from a PCA of leaf, stem, and root percent N and C:N ratios).

(Model 4), significantly predicted total exudation rate ($p = 0.009$, estimated $R^2 = 0.12$, Figure 5a) and marginally predicted mass-specific exudation ($p = 0.02$, estimated $R^2 = 0.35$).

Although tissue nitrogen varied with treatment, potentially due to nitrogen limitation in the low nitrogen treatments, I found no relationship between total exudation and either principal component of tissue nitrogen traits (Model 5). For mass-specific exudation, however, I observed a significant relationship with the first nitrogen principal component, such that exudation increased with tissue C:N ratio and decreased as leaf and root percent N increased ($p = 0.0002$, estimated $R^2 = 0.34$, Table 4, Figure 5b).

Using Akaike weights calculated separately for the total exudation models and the mass-specific models, I assessed the relative support for the five models described

above (1. light & nitrogen treatment, 2. size traits, 3. leaf photosynthesis, 4. whole-plant photosynthesis, and 5. tissue nitrogen traits). Of these models, the size PCA model is most likely to best predict both total and mass-specific exudation (for both $w = 0.99$, Table 4). Consistent with the Akaike weights analysis, the size PCA models explained the largest amount of variation in both total and mass-specific exudation (estimated $R^2 = 0.52$ and 0.48, respectively; Table 4).

1.3.4 Species Variation

The species-only model explained a small portion of the variation in total exudation (estimated $R^2 = 0.14$) and a moderate amount of variation in mass-specific exudation ($R^2 = 0.38$). Several of the traits I measured may partially contribute to these numbers.

Size: When I remove species from the size traits model for total exudation, the estimated R^2 drops to 0.26 from 0.52. It is likely that size contributes to species' variation in exudation (Figure 4). However, the traits-plus-species model for total exudation has a greater- than-additive increase in estimated R^2 from the species- and traits-only models, suggesting that additional traits not represented in this model interact with size to contribute to species differences in total exudation. For mass-specific exudation, the estimated R^2 values from the species-only and size traits-only models are each substantial (0.38 and 0.31, respectively), and they contribute less-than-additively to the combined estimated R^2, indicating that size traits explain a portion of the species effect on mass-specific exudation.

Photosynthesis: Leaf-level photosynthesis was not a significant predictor of exudation. For total exudation, whole-plant photosynthetic rate (estimated $R^2 = 0.17$, Figure 5a) may capture much of the variation explained by the species (estimated $R^2 =$

0.14), although the combined model explains little variation in total exudation (estimated $R^2 = 0.12$). For mass-specific exudation, the combined model explains a more substantial amount of variation (estimated $R^2 = 0.39$), but most of this relationship seems to be due to the effect of species (estimated $R^2 = 0.38$) and not whole-plant photosynthetic rate (estimated $R^2 = 0.09$).

Tissue Nitrogen: Tissue nitrogen is not a significant predictor of and does not explain much variation in total exudation (Table 4). For mass-specific exudation, the first nitrogen principal component (estimated $R^2 = 0.23$) captures a substantial portion of the variation explained by species (estimated $R^2 = 0.38$) and by the combined model (estimated $R^2 = 0.34$). This result indicates that tissue N traits may contribute to species differences in mass-specific exudation (Figure 5b).

Based on these results, I integrated the traits identified above into two additional models to assess whether they additively account for species effects on exudation: (1) Total exudation as a function of the first size principal component and whole-plant photosynthetic rate and (2) mass-specific exudation as a function of the size and tissue nitrogen first principal components. However, these models explain no more variation than the size models alone (estimated $R^2 = 0.25$ and 0.34, respectively, Table 4). Although whole-plant photosynthetic rate explained a small amount of variation in total exudation and tissue nitrogen explained some variation in mass-specific exudation, these traits do not enhance the variation explained by size traits.

1.4 Discussion

Although I found no clear effect of light or nitrogen treatments on exudation rates (Hypothesis 1), traits (whole-plant photosynthetic rate and tissue N) were significantly related to exudation rates (Hypotheses 2B & 2C). Consistent with the lack of treatment

effect, these relationships between exudation and both photosynthesis and tissue N were not strong. Both total and mass-specific exudation rates were most strongly predicted by plant size (Hypothesis 2A). The variation explained by these three traits appears to explain some of the relationship between species and exudation (Hypothesis 4). This result supports my approach of measuring specific traits to predict exudation behavior.

1.4.1 Environment and Exudation

Previous research demonstrates clear effects of environmental conditions such as light and nitrogen on root carbon exudation rates. Thus, I was surprised to find neither an effect of light or nitrogen levels on exudation rates nor an interaction between these factors (Hypothesis 1). In this study, I limited the duration of the low light and nitrogen treatments in order to isolate carbon and nitrogen limitation from growth effects, while most studies of nitrogen availability involve chronic nitrogen deprivation. Although the low nitrogen treatment significantly reduced tissue nitrogen concentrations, nitrogen limitation may substantially affect carbon exudation only with longer deficiency, potentially mediated by the effects of nitrogen on root growth.

In the low-light, low-nitrogen treatment, I observed non-significantly lower total exudation rates than under the other treatments. These patterns hint at light and nitrogen influences on exudation and might be more overt with more statistical power or longer treatment duration. I also observed non-significant trends in the relationship between total exudation and plant size, such that in several species in the low-light, low-nitrogen treatment, total exudation appeared to decrease as size increased (Appendix A). If this pattern holds, it would suggest a curious interaction between

environmental limitations and plant size, with larger plants curtailing exudation more strongly in low-resource environments.

1.4.2 Traits and Exudation

Plant size was strongly related to both total and mass-specific exudation (Hypothesis 2A), and this relationship overshadowed the effects of photosynthesis and tissue N. That size was strongly related to total exudation is virtually self-evident: larger root systems have more capacity to exude. (However, within *P. virgatum* I found little increase in total exudation with size.) That mass-specific exudation generally decreased across species as plants got larger does not seem to be due to changes in the ratio of mass to surface area as plants get larger: I see the same relationship with exudation per unit surface area (data not shown). Others have found that older plant roots exude less per unit root mass than do younger plant roots (described in Kuzyakov 2002). The plants in this study were all the same age, and thus larger plants should have more young roots, grown recently as plants expanded their root systems. Therefore, I might have expected the opposite pattern overall, as I observed within *M. vimineum.*

I clustered together size traits that are collinear with each other but that capture slightly different characteristics. I might expect that specific root size traits such as total root length or total surface area would be stronger predictors of exudation, because these traits indicate contact between root and soil. However, in these data, overall size appears to be a more critical predictive metric (Appendix B). When I examine size traits individually, either the size principal components model has stronger support (total exudation) or Akaike weights and estimated R^2 values provide conflicting support for different root size traits (mass-specific exudation). Due to the size of these root systems, I did not analyze root tip density or branching frequency, traits which may

offer more detailed insight into exudation patterns (McDougal and Rovira 1970, Badri and Vivanco 2009).

My results suggest only a minor relationship between photosynthesis and root carbon exudation within and across species (Hypothesis 2B). I expected carbon supply to influence carbon released from roots, as has been found in multiple previous studies within species (Whipps 1984, Hodge et al. 1997, Dilkes et al. 2004, Hill et al. 2007, Phillips et al. 2009, Phillips et al. 2011). Further, species' differing baseline leaf photosynthetic rates did not contribute to their variation in mass-specific exudation. In light treatment experiments, long-term high light treatments should enhance growth relative to low light treatments; this high growth rate should elevate nutrient demand over low light environments and may induce high light plants to enhance carbon exudation in order to mobilize more nutrient sources. Such longer-term pathways may have a larger impact on exudation rates than do photosynthesis and carbon supply directly, which my study focused on. Further, although carbon supply has generally been found to influence carbon exudation, some additional studies have found little effect of shading on exudation (Personeni et al. 2007), similar to this work, suggesting that the relationship between carbon supply and exudation is complex.

Mass-specific exudation decreased as root and leaf tissue N increased. Although tissue N decreased with the low N treatment, the relationship between tissue N and with mass-specific exudation appears to be due more to species differences in tissue N (Figure 5b). This finding is unusual: I expected N limitation, and thus N demand, to increase exudation whereas it appears that species with greater N use efficiency (higher C:N ratio), and theoretically less N demand, exuded more carbon on a per-mass basis. Plants with high C:N ratios may be more likely to have extra C that they can afford to

exude. Tissue N did not drive exudation in this study—it appeared to be redundant with and less powerful than biomass as a predictor—but the relationship between tissue nutrient status and exudation merits further investigation.

1.4.3 Species and Exudation

My results support trait-based analyses of species functional differences, including for carbon exudation. Three measured traits—plant size, whole-plant photosynthetic rate, and tissue N concentration—appeared to capture a portion of the variation in exudation across species. However, these traits did not cumulatively explain all species' variation, nor did they have additive explanatory power: All of the variation captured by whole-plant photosynthetic rate and tissue nitrogen was also captured by size. For whole-plant photosynthetic rate, this result makes sense: I calculated this trait by multiplying leaf photosynthetic rate by a size trait (total leaf area). Tissue nitrogen concentration could be indirectly collinear with size as well. To enhance our ability to predict relative exudation rates across species, I suggest further investigation of cross-species trait relationships with exudation rates. Which traits create the interaction between size and species in predicting exudation? In this study, I did not measure root tip density, and such root morphological characteristics may also be important.

1.4.3 Limitations

The hydroponic incubation method I used to collect root exudates allowed us to measure exudation on relatively large, mature individuals, but this method has limitations (Jones 1998, Neumann et al. 2009). Hydroponic solutions do not mimic the physical properties of soil. Further, disturbance to plants can affect their exudation patterns. Although the plants had over three weeks to adjust after their initial

transplantation to hydroponic media, transferring the plants to the carbon-free nutrient solution immediately before the incubation involved some disturbance of both roots and shoots. Further, I cannot distinguish exudation from other forms of rhizodeposition, estimate microbial respiration of exuded carbon, or estimate re-uptake by plants of exuded carbon (Meharg and Killham 1991, Jones and Darrah 1993, Nguyen 2003). High solution temperatures may have additionally facilitated quick decomposition of exudates, as solutions were exposed to air temperatures ranging from 26ºC to 35ºC. Exudation itself may or may not have been influenced by the solution temperature (Rovira 1969, Hill et al. 2007). My measured values may underestimate total exudation.

1.4.4 Implications for Soil Carbon Cycling

Our species results have implications for carbon cycling in wetland and agricultural areas. *Microstegium vimineum* had the highest total and widest range of exudation rates, and it was the only species to have mass-specific exudation increase with mass. *M. vimineum* is an invasive plant from Asia growing in high densities in riparian and bottomland areas throughout the southeastern United States. Previous research has demonstrated that *M. vimineum* can enhance decomposition of native soil organic matter soil, resulting in soil carbon loss (Strickland et al. 2010, Strickland et al. 2011). My results showing comparatively high rates of carbon exudation from *M. vimineum*, including an increase in mass-specific exudation as plants get larger, suggest one potential mechanism behind the previous findings. Although the role of exudates in soil carbon turnover and sequestration is not yet well understood, labile root exudates can stimulate decomposition of more recalcitrant soil organic matter (Kuzyakov 2002, Chapin et al. 2009, de Graaff et al. 2010). High levels of labile carbon compounds exuded from *M. vimineum* roots could potentially prime decomposition of soil organic

matter at rates higher than the native species compared in this study, leading to soil carbon loss.

Another research species, *Panicum virgatum*, is being substantially investigated as a biofuel crop. With agricultural use of *P. virgatum* expanding in the United States, understanding its impacts on soil carbon cycling is increasingly relevant, especially as perennial crops including *P. virgatum* specifically may help sequester soil carbon (Garten and Wullschleger 2000, Anderson-Teixeira et al. 2009, Collins et al. 2010). My work demonstrates that despite high rates of photosynthesis, *P. virgatum* may generally exude carbon at a low rate, potentially with little increase in total exudate release as plants increase in size. (Although individuals in my research reached reproductive maturity and flowered, *P. virgatum* individuals were small relative to fully-sized field-grown individuals; my result may not extend to individuals substantially larger than the plants in this study.) This finding has implications for *P. virgatum*'s effects on the soil microbial community, soil decomposition rates, and soil carbon sequestration which merit further investigation. By exuding at low levels, *P. virgatum* may not support substantial exudate-primed decomposition of recalcitrant soil organic matter, potentially leading to greater soil carbon storage than under crops than exude labile carbon at higher levels.

1.4.5 Conclusions

The effects of environmental factors such as light and nitrogen on carbon exudation remain uncertain. Although results elsewhere in the literature demonstrate responses of exudation rates to light and nitrogen conditions, my results demonstrate that these effects are not universal. Additional conditions may interact with these environmental factors and plant traits to produce the demonstrated effects. My results further illustrate that specific functional traits such as size and tissue N concentration

contribute to species variation in exudation rates, including mass-specific exudation. Additional traits must also contribute to the remaining species effect, but my data do not suggest that photosynthetic variation strongly influences species' differences in exudation. A more detailed trait-based approach will hopefully allow us to more accurately predict trends in exudation across species and constrain the functional impacts of species on soil carbon and nutrient cycling. For this goal, we need further research not only into the functions of root exudates but also the mechanisms that underlie variation in exudation across individuals and species.

Appendix A

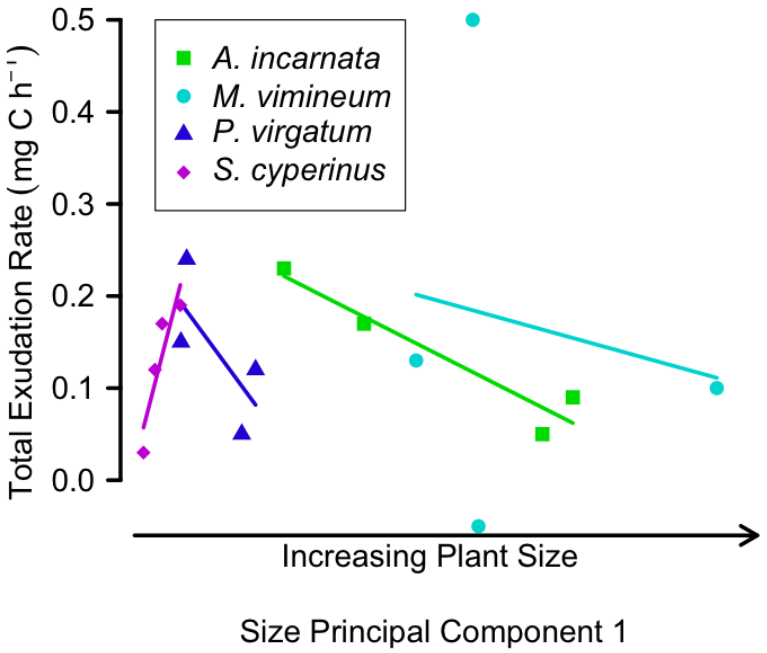

Figure A1: Total exudation rate as a function of size and species in the low light, low nitrogen (LLLN) treatment. Size is represented as the first principal component of a PCA of shoot mass, leaf area, root mass, root length, and root volume. Non-significant trends hint at total exudation decreasing as plant size increases in this treatment.

Appendix B

Table B1: Models of individual size traits with their AIC values, estimated R^2 values, Akaike weights, significant variables and p-values, where ‡ ≤ 0.10, † ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, and **** ≤ 0.0001. Interaction terms are represented by the two individual terms separated by the symbol *. Akaike weights were calculated with these models and models 1 to 5 above. For both total and mass-specific exudation, the root mass model has a high *w* value, and with mass-specific exudation it may be the strongest model overall. However, estimated R^2 values suggest that other root size traits are comparable in explaining variation in mass-specific exudation.

	Y = Total exudation					*Y = Mass-specific exudation*				
Model	**AIC**	**Est. R^2**	**w_i**	**Significant terms**	**p-values**	**AIC**	**Est. R^2**	**w_i**	**Significant terms**	**p-values**
Shoot Mass									Shoot.mass	****
Y = Shoot.mass*Sp + B	70.1	0.23	6.5 e-09	AGB	***	7.2	0.44	2.2 e-05	Species	**
									Shoot.mass * Species	†
Leaf Area									Leaf.area	****
Y = Leaf.area*Sp + B	116.5	0.12	5.5 e-19	Leaf.area	**	48.7	0.43	2.2 e-14	Species	***
									Leaf.area * Species	†
Root Mass				BGB	****				Root.mass	****
Y = Root.mass*Sp + B	34.9	0.44	0.29	Species	†	-14.2	0.48	0.99	Species	*
				BGB * Species	***				Root.mass * Species	**
Root Length									Root.length	****
Y = Root.length*Sp + B	67.6	0.31	2.3 e -08	Root.length	****	5.1	0.51	6.4 e-05	Species	***
									Root.length * Species	***
Root Area				Root.area	****				Root.area	****
Y = Root.area*Sp + B	97.1	0.39	9.0e-15	Species	‡	40.2	0.51	1.5 e-12	Species	**
				Root.area * Species	‡				Root.area * Species	***
Root Volume				Root.volume	***				Root.volume	****
Y = Root.volume*Sp + B	56.1	0.40	7.2 e -06	Species	**	1.5	0.50	3.9 e-04	Species	**
				Root.volume * Species	**				Root.volume * Species	***

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