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# Intraspecific variability and reaction norms of forest understory plant species traits

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## Summary

1. Trait-based models of ecological communities typically assume intraspecific variation in functional traits is not important, though such variation can change species trait rankings along gradients in resources and environmental conditions, and thus influence community structure and function.
2. We examined the degree of intraspecific relative to interspecific variation, and reaction norms of 11 functional traits for 57 forest understory plant species, including: intrinsic water-use efficiency (iWUE), $\Delta^{15}N$, 5 leaf traits, 2 stem traits and 2 root traits along gradients in light, nitrogen, moisture and understory cover.
3. Our results indicate that interspecific trait variation exceeded intraspecific variation by at least 50% for most, but not all traits. Intraspecific variation in $\Delta^{15}N$, iWUE, leaf nitrogen content and root traits was high (47-70%) compared with most leaf traits and stem traits (13-38%).
4. $\Delta^{15}N$ varied primarily along gradients in abiotic conditions, while light and understory cover were relatively less important. iWUE was related primarily to light transmission, reflecting increases in photosynthesis relative to stomatal conductance. Leaf traits varied mainly as a function of light availability, with some reaction norms depending on understory cover. Plant height increased with understory cover, while stem specific density was related primarily to light. Resources, environmental conditions and understory cover did not contribute strongly to the observed variation in root traits.
5. Gradients in resources, environmental conditions and competition all appear to control intraspecific variability in most traits to some extent. However, our results suggest that

species cross-over (i.e., trait rank reversals) along the gradients measured here are generally not a concern.

6. Intraspecific variability in understory plant species traits can be considerable. However, trait data collected under a narrow range of environmental conditions appears sufficient to establish species rankings and scale between community and ecosystem levels using trait-based models. Investigators may therefore focus on obtaining a sufficient sample size within a single set of conditions rather than characterizing trait variation across entire gradients in order to optimize sampling efforts.

7. **Key-words** functional traits, herbaceous layer, Pacific Northwest, stable isotopes, water use efficiency

## Introduction

Trait-based models of ecological communities are used increasingly in community ecology because they promise greater generality, predictive power, and ability to scale between community and ecosystem levels of organization (McGill et al. 2006, Suding et al. 2008). Large collaborative databases composed of average species trait values now facilitate the large-scale adoption of trait-based approaches (e.g., Wright et al. 2004, Kattge et al. 2011). However, the use of species averages may discount the importance of intraspecific variation in community assembly processes, species co-existence and associated ecosystem functions (Bolnick et al. 2011, Laughlin et al. 2012, Violle et al. 2012, Hart, Schreiber & Levine 2016). Empirical studies are therefore necessary to evaluate the degree of intraspecific variation in traits, and to determine whether intraspecific variation may improve or modify trait-based models of species assemblages (Siefert et al. 2015, Shipley et al. 2016).

The mass ratio theory posits that the relative contribution of species in a community is proportional to its contribution to primary production, and that ecosystem processes are determined by the traits of dominant plant species (Grime 1998). Mass ratio theory provides the basis for using community-aggregated traits to model responses of plant communities to variation in the environment and effects of community composition on ecosystem processes and/or services. Species average trait values are commonly weighted by their relative abundances and summed to calculate community aggregated traits (Pérez-Harguindeguy et al. 2013). This is considered sound when interspecific variability exceeds intraspecific variability, or when species rankings are maintained across gradients in resources and environmental conditions (Fig. S1a in Supporting Information; Garnier et al. 2001, Kazakou et al. 2014). Conversely, trait reaction norms (i.e., responses of traits to gradients in resources and environmental conditions) may result in species cross-over, here defined as shifts in rankings of species traits along gradients in resources or competition (*sensu* Givnish et al. 2004; Fig. S1b). Species cross-over could result in different community aggregated trait values along those gradients for the same community and/or differences in species composition for a given community aggregated trait value – requiring researchers to account for intraspecific variation when calculating community aggregated traits. There is evidence for cross-over in the physiological performance among coexisting and/or closely related species across environmental gradients (Chazdon 1992, Kaelke et al. 2001, Givnish, Montgomery & Goldstein 2004). Traits are considered proxies for physiological performance. However, despite the increasing use of trait-based approaches, the assumption that species trait rankings are constant across gradients in resources, environmental conditions and competition has been evaluated sparingly (Garnier et al. 2001, Albert et al. 2010, Auger & Shipley 2013, Lepš et al. 2011, Kazakou et al. 2014). More intensive sampling within species to account for intraspecific trait variability and cross-over may improve trait-based models of plant communities, but likely comes at a cost to the number of species that can be sampled when resources are limited (Paine, Baraloto & Diaz 2015).

Trait-based approaches are rapidly being adopted to study effects of disturbance, forest management, climate change and interactions thereof on understory plant communities and associated ecosystem services (Neill & Puettmann 2013, Kern et al. 2014, Sabatini et al. 2014, Sonnier et al. 2014). Forest understory plant communities in the temperate zone typically contain 2-20+ times the number of species as the overstory (Gilliam 2007). Understory plant species are sensitive indicators of resources and environmental conditions (Daubenmire 1976), and may be partitioned along gradients in soil properties including moisture and nutrients, light transmission as it relates to overstory tree structure, and climate (Ares, Berryman & Puettmann 2009, Burton et al. 2011, 2014). Similarly, understory species physiological performance, reflected in morphological and physiological traits, is likely to vary along these gradients (McGill et al. 2006). Recent meta-analyses show that intraspecific variation in whole-plant traits is greater than biochemical traits, which exceeds intraspecific variation in morphological traits (Siefert et al. 2015). To date, much research has focused on adaptation and acclimation of leaf traits and associated physiological processes to shade (e.g., Givnish 1988, Chazdon 1992, Ellsworth and Reich 1992 and 1993, Kaelke et al. 2001, Givnish, Montgomery & Goldstein 2004), yet little empirical work exists for temperate understory species. Intraspecific variation in leaf traits along gradients in soil properties, and climatic conditions, and effects of interactions among these gradients, are even less well understood (e.g., Roche, Díaz-Burlinson & Gachet 2004, Nicotra et al. 2010, Funk et al. 2016). Moreover, little is known about how whole-plant (e.g., water use efficiency, nitrogen use strategy), stem (e.g., plant height, stem specific density) and root traits (e.g., specific root length, rooting depth) vary along these gradients within species.

Our goal was to investigate the assumptions underlying the common practice of using species means in trait-based modeling of plant communities. We examined intraspecific relative to interspecific trait variability. Additionally, we assessed alternative models of trait reaction norms and cross-over along specific gradients in light, soil nitrogen, understory cover and climatic conditions using hierarchical mixed models. All models include random effects accounting for the nested structure of the sampling design. We do not control for the effects of local adaptation or genetic variation, which may vary along environmental and resource gradients with traits (e.g., Ravenscroft, Fridley & Grime 2014). We evaluated the hypotheses that trait variation among species exceeds variation within species, and species maintain rankings along environmental and resource gradients. Finally, we interpreted trait reaction norms considering expected physiological responses (Table 1). We assessed a suite of leaf, stem and root traits for plant species found in the understory of Douglas-fir forests in western Oregon. We also examined whole-plant traits including intrinsic water use efficiency based on stable carbon ($\delta^{13}C$) isotopes (Brooks et al. 1997, Foster & Brooks 2005), and nitrogen (N) stable isotope discrimination relative to soil ($\Delta^{15}N$) - a metric of niche partitioning in nitrogen use strategies among plants (Nadelhoffer et al. 1996, Gubsch et al. 2011).

## Materials and methods

### STUDY AREA

We collected functional trait data at seven sites located in western Oregon Coast Range and western Oregon Cascades, USA. These sites are the locations of a replicated manipulative experiment known as the Density Management Study. For more detailed information about experiment, history, soils, and climate of the study sites, see (Cissel et al., 2006). The sites are distributed across the western hemlock zone (Franklin and Dyrness 1988) covering a broad geographic (sites range between 10 and 245 km apart) and climatic gradient (across sites average 2001-2010 mean annual temperatures range from 8.6-11.7 °C, mean annual precipitation ranges from 1274-2080 mm; Wang et al. 2012). The climate is Mediterranean with mild, wet winters and warm, dry summers (Cissel et al. 2006). Soils are well- to poorly-drained (highly weathered) Ultisols and (younger, less structured) Inceptisols, and vary widely among sites in nitrogen (N) availability (Thiel & Perakis 2009). Forests were thinned ~60- to 80-year-old Douglas-fir (*Pseudotsuga menziesii*) stands with varying abundances of western hemlock on some sites. Other conifer species, such as western redcedar (*Thuja plicata*), and hardwood species including bigleaf maple (*Acer macrophyllum*), red alder (*Alnus rubra*), Pacific dogwood (*Cornus nutalli*), Pacific madrone (*Arbutus menziesii*), and golden chinquapin (*Chrysolepis chrysophylla*) were minor components of the overstory.

The Density Management Study uses a randomized complete block design with one replicate of four density treatments at each of seven 94 – 131 ha sites (Cissel et al. 2006). This experimental structure ensured a broad gradient of overstory structures and associated resources and environmental conditions for the understory plants (S1 in Supplementary Information). We used overstory and understory data collected from permanent plots to select dominant understory species and characterize local overstory conditions (Appendix S1 in Supporting Information).

TRAIT DATA

We focused on a suite of ten whole plant, leaf, stem and root traits commonly used to infer ecological strategies of plants (Table 1). Foliar stable isotopes (δ) for C and N provide information about ecological strategies at the whole plant level. Higher values of foliar C isotope ($\delta^{13}C$) generally indicate higher intrinsic water use efficiency of plants (iWUE, $A/g_s$), which may be sensitive to variation in light, soil moisture and microclimatic conditions in the forest understory (Farquhar et al. 1989, Farquhar, Ehleringer & Hubick 1989, Ehleringer 1991). Foliar N isotopes ($\delta^{15}N$) are influenced by many aspects of the environmental physiology of plant N uptake including N form(s) used (i.e., inorganic; $NO_3^-$ vs $NH_4^+$ vs. organic N), timing and depth of N uptake, as well as mycorrhizal influence and within-plant N partitioning. These many factors, their potential interactions, and their environmental dependence complicate attempts to resolve specific cause(s) of high vs. low plant $\delta^{15}N$ values in natural settings (Evans 2001). However, in general wider variation in $\delta^{15}N$ values broadly reflects greater diversity in plant N use strategies (Nadelhoffer et al. 1996, Gubsch et al. 2011). Leaf traits, including specific leaf area (SLA, $mm^2/mg$), leaf nitrogen content (LNC, mg/g), leaf nitrogen per area ($N_{area}$, $g/m^2$), leaf size ($cm^2$), and leaf dry matter content (LDMC, mg/g), indicate major leaf economic trade-offs between high rates of resource acquisition and resource conservation (Diaz et al. 2004, Wright et al. 2004, Pierce et al. 2016). We also measured stem traits, including vegetative height, as an indicator of a potential tradeoff between height growth and photosynthetic and conductive tissues maintaining water transport (Givnish 1982, Givnish 1995); and stem specific density (SSD, $mg/mm^3$), as an indicator of a potential trade-off between growth, and strength and decay resistance (Chave et al. 2009). Rooting depth (cm) can affect resource acquisition and persistence (e.g., Antos & Halpern 1997) while specific root length (SRL, m/g), defined as the ratio of a standard unit of resource acquisition (root length) to the resource investment (mass), is positively related to rates of nutrient and water uptake and relative growth rate, but negatively related to root lifespan (Eissenstatt 1991). Plants are therefore expected to exhibit variation in these traits in response to variation in resources and environmental conditions (Table 1).

*Field data collection*

We measured traits of all understory plant species comprising ≤ 80% of the cumulative importance (the average of the relative frequency and relative abundance, measured here as percent cover) at each site following standard protocols, with modifications for specific root length (Pérez-Harguindeguy et al. 2013, see Appendix S1 in Supplementary Information for details). Plant samples were collected in 2015, three to five years following a second experimental treatment. At each permanent vegetation survey plot, individual plants located closest to plot center were sampled provided they were not severely suppressed by other understory vegetation and did not exhibit signs of damage due to e.g., herbivory or diseases. Plant height and rooting depth were measured *in situ* (Appendix S1). Once all *in situ* morphological measurements were recorded, we collected each specimen for processing in the laboratory (Appendix S1). Plant samples were stored in a 3ºC dark cold storage room until processed in the laboratory.

*Calculating plant intrinsic water use efficiency and leaf $\Delta^{15}N$*

Soil $\delta^{15}$N values in forests vary widely in response to local topography, N fixation, and disturbance history, which can contribute to variation in plant tissue $\delta^{15}$N among sites (Perakis, Sinkhorn & Compton 2011, Perakis, Tepley & Compton 2015). To control for background soil variation and enable comparisons of $\delta^{15}$N as a trait-based measure of plant N use, we calculated

$\Delta^{15}N$ as $\delta^{15}N_{soil}$ (measured in each plot) subtracted from leaf $\delta^{15}N$ prior to statistical analysis (e.g., Gubsch et al. 2011).

$$\Delta^{15}N = \delta^{15}N_{leaf} - \delta^{15}N_{soil} \tag{2}$$

From foliar $\delta^{13}C$ values, we calculated iWUE (Farquhar et al. 1989a, Farquhar et al. 1989b, Ehleringer 1991). The $\delta^{13}C$ values in plant leaves are influenced by the variation in the atmospheric $CO_2$ isotopic composition ($\delta^{13}C_{air}$), and by biophysical and biochemical processes in the plant. To calculate iWUE, we first calculated carbon isotope discrimination ($\Delta^{13}C$) to account for variation due to $\delta^{13}C_{air}$ (Farquhar, O'Leary & Berry 1982):

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}/1000} \tag{3}$$

We estimated $\delta^{13}C_{air}$ for each individual sample using the model of Buchmann, Brooks & Ehleringer (2002) because $\delta^{13}C_{air}$ varies vertically within forest understories as a result of respired $CO_2$ and low wind speeds:

$$\delta^{13}C_{air} = \delta^{13}C_{trop} - \frac{0.023 * L}{h} \tag{4}$$

where $L$ = leaf area index (LAI), $h$ = height and $\delta^{13}C_{trop}$ is $\delta^{13}C$ of the troposphere, well above the influence of the canopy. We estimated LAI from hemispherical photos taken at plot centers (described below). Field measurements of plant height were used for $h$. We estimated $\delta^{13}C_{trop}$ during the 2015 growing season to be -8.55‰ using a simple linear regression of growing season $\delta^{13}C_{trop}$ on year using Mauna Loa data from 1990-2014 ($r^2 = 0.98$).

We then used $\Delta^{13}C$ values to estimate iWUE [i.e., photosynthesis ($A$), divided by stomatal conductance ($g_s$)] using the relationship between $\Delta^{13}C$ and the ratio of internal $CO_2$ to atmospheric $CO_2$ ($c_i/c_a$) described by Farquhar et al. (1989):

$$\Delta^{13}C = a + (b - a)\left(\frac{c_i}{c_a}\right) \tag{5}$$

where $a$ = diffusion (4.4‰), b = RuBisCO ~29‰, $c_i$ and $c_a$ are internal and ambient $CO_2$, respectively. iWUE can then be estimated from $c_i$ and $c_a$ as follows:

$$\mathrm{iWUE} = \frac{A}{g} = \frac{c_a - c_i}{1.6} \quad (6)$$

where A is the rate of photosynthesis (μmol $m^{-2}$ $s^{-1}$), g is stomatal conductance (mmol $m^{-2}$ $s^{-1}$), 1.6 is the ratio of diffusivities of water and $CO_2$ in air and $c_a$ is predicted to be 400 parts per million using a simple linear regression of growing season $CO_2$ on year using Mauna Loa data from 1969-2014 ($r^2$ = 0.998).

RESOURCES AND ENVIRONMENTAL CONDITIONS

To characterize local light transmission and associated environmental conditions (impacts on humidity and temperature) in the understory, we took hemispherical photographs using a Nikon Coolpix 5000 digital camera and FC-E8 fisheye lens adapter. Photos were taken at plot centers after mounting and leveling the camera on a tripod at 1 m height under variable weather conditions (i.e., sunny as well as cloudy skies). Hemispherical photos were analyzed for leaf area index (LAI) and Gap Light Index (GLI) using Hemisphere v. 2.16, © Patrick Schleppi, WSL. Additionally, we estimated total vascular plant cover (percent) within a 2.5 m radius of the specimen as a proxy measure for understory competition intensity (Wagner & Radosevich 1998). We collected one mineral soil sample in the center of each plot using a 5.8 cm diameter corer to a depth of 13 cm, sieved samples through a 2 mm sieve, and ground and analyzed samples for total C and N, $\delta^{13}C$, and $\delta^{15}N$ as described above. To understand the integrated effects of temperature and precipitation on plant traits, we used annual climatic moisture deficit (CMD) calculated as the sum of the monthly differences between potential evapotranspiration and precipitation (Wang et al. 2012). Annual CMD data (2001-2010) were obtained for all plots based on geographic coordinates and elevation from down-scaled spatial interpolations of monthly data, accounting for effects of local topography, coastal influences and temperature inversions using ClimateWNA (Wang et al. 2012).

## DATA ANALYSIS

We analyzed the trait data using linear mixed effects models to account for the hierarchical sampling design. First, we analyzed intercept-only models of traits with random effects to quantify the 1) variation associated with differences among species $\sigma^2_{species}$ and 2) variability within species across sites (sites nested in species, $\sigma^2_{sites}$). Residuals therefore represent intraspecific variability within sites ($\sigma^2_{resid}$). To assess whether intraspecific variability was on average lower than interspecific variability, we examined the ratio of the variance associated with interspecific variation ($\sigma^2_{species}$) to the total variance associate with intraspecific variation ($\sigma^2_{sites} + \sigma^2_{resid}$).

Then we analyzed a series of alternative models composed of fixed and random effects. Fixed effects accounted for the effects of resources and environmental conditions that vary over progressively broader spatial and temporal scales on observed trait values, including understory cover, gap light index (GLI), soil nitrogen (N) and moisture deficits. We also treated species as a fixed effect to be able to sort out the roles of resources and environmental conditions after accounting for differences among species, and to examine the importance of interactions between species and resource/environmental variables potentially resulting in cross-over. In this context, the random effects structure accounts for the hierarchical experimental design (i.e., site, treatment nested in site and plot nested within sites were treated as random intercepts).

For each trait, we used a multi-step modeling process to compare a sequence of alternative models comprised of progressively more variables (Table S1 in Supplementary Information). In step one, we fit null, intercept-only models consisting of random effects only. In step two, we added the effect of species. In step three we added variables describing effects of fine scale variation of overstory and understory vegetation structure on resources (e.g., light, soil moisture, available nitrogen) indexed by GLI and understory cover, and plausible interactions. In step four, we considered intermediate-scale variation in soil nitrogen (total) and plausible interactions with variables selected in steps two and three (i.e., species, GLI and understory cover) were assessed. In step five, we assessed alternative models integrating the best model selected in previous steps with additional effects of broad-scale variation in climatic moisture deficit (CMD) and plausible interactions. We tested for species cross-over along all environmental/resource gradients by comparing alternative models with and without interactions between species and the gradient variable of interest. For each step, we evaluated alternative models consisting of various plausible combinations of variables and appropriate two-way interactions using AICc, a bias-corrected version of the Akaike Information Criterion (AIC) for small sample sizes (Burnham & Anderson 2002). Three-way interactions were considered when the categorical effect for species was one of the three terms. The best model was selected and carried forward to the next step; if top-ranking models did not differ substantially ($\Delta$AICc < 2) we used the model with the fewest parameters. We parameterized the best model from all steps, and plotted marginal predictions of species traits along selected gradients to interpret reaction norms in light of hypotheses in Table 1.

For each alternative model, we estimated the variance explained by the marginal fixed effects alone ($R^2_m$), and by the fixed effects conditioned on random effects ($R^2_c$) (Nakagawa &

Schielzeth 2013). Changes in $R^2_c$ and $R^2_m$ following the addition of fixed effects reflected the importance of that effect to explaining variation in traits. Additionally, to compare the relative importance of variables, we calculated semi-partial $R^2$s following Edwards et al. (2008) for all fixed effects in the selected models. Semi-partial $R^2$ values ($R^2_\beta$) measured the marginal contributions of predictor variables conditioned on other predictor variables in the models. All analysis was done with SAS version 9.4, using the mixed procedure (SAS Institute, © 2002-2012).

## Results

INTER- AND INTRASPECIFIC TRAIT VARIABILITY

All whole plant, leaf, stem and root traits exhibited considerable levels of inter- and intra-specific variability (Fig. 1). Intraspecific variation was highest for whole plant measures (the ratio of interspecific to intraspecific variation for $\Delta^{15}N$ = 0.38 and iWUE = 0.47), followed by root traits (interspecific:intraspecific for specific root length, SRL, = 0.89, rooting depth = 0.50). With the exception of mass-based leaf nitrogen content, LNC, (interspecific:intraspecific = 0.79), intraspecific variation was lowest for leaf traits (interspecific:intraspecific for SLA = 2.67, nitrogen per unit area, $N_{area}$ = 1.58, LDMC =3.06, and leaf size = 6.53) and stem traits (interspecific:intraspecific for height = 3.06, SSD = 1.94). These results are largely consistent with those of Siefert et al. (2015).

TRAIT REACTION NORMS

*Whole plant traits*. – The selected model indicated that $\Delta^{15}N$ was related to differences among species (semi-partial $R^2_\beta$ = 0.37), understory cover ($R^2_\beta$ = 0.02), soil N ($R^2_\beta$ = 0.15) and climatic moisture deficit (CMD; $R^2_\beta$ = 0.30) but not gap light index (GLI). Specifically, plant $\Delta^{15}N$ (i.e., $\delta^{15}N_{plant}$ normalized to $\delta^{15}N_{soil}$) decreased with understory cover and increased with soil N and CMD (Fig. 2; Table 2). These fixed effects explained 35% of the variation ($R^2_m$), with an additional 12% explained by random effects ($R^2_c$ = 0.47).

In contrast, the best supported model for intrinsic water use efficiency (iWUE) showed that in addition to being related to differences among species ($R^2_\beta$ = 0.54), iWUE increased with GLI ($R^2_\beta$ = 0.36), and decreased with understory cover ($R^2_\beta$ = 0.02) and soil N ($R^2_\beta$ = 0.05; Fig. 3). Fixed effects accounted for the majority of variation explained for iWUE ($R^2_m$ = 0.49, $R^2_c$ = 0.54; Table 2).

*Leaf traits*. – Variation in leaf traits was related primarily to differences among species ($R^2_\beta$ ranges 0.56-0.88), GLI, and understory cover (Fig. 3, Table 2). SLA ($R^2_\beta$ = 0.11), LNC ($R^2_\beta$ = 0.06), and leaf size ($R^2_\beta$ = 0.27) decreased, while leaf dry matter content (LDMC; $^2_\beta$ = 0.12) and $N_{area}$ ($R^2_\beta$ = 0.29) increased with GLI. Relationships of these traits to understory cover were relatively weak and in the opposite direction, as for GLI (Figs. S2 in Supplementary Information). The model for SLA ($\Delta AIC_c$ = 0.5) and LDMC also included an interaction between understory cover and GLI ($R^2_\beta$ = 0.01 and 0.04, respectively). Additionally, $N_{area}$ was negatively related to soil N ($R^2_\beta$ = 0.06; Fig. S3 in Supplementary Information). Fixed effects explained between 53 and 87% of the variation in leaf traits ($R^2_m$), with random effects explaining a relatively small amount (1-6%, $R^2_c$, Table 2).

*Stem traits*. – Variation in stem traits was related to variation among species, and in understory cover and GLI. Plant height varied among species ($R^2_\beta$ = 0.77) and increased with understory cover ($R^2_\beta$ = 0.19). Stem specific density (SSD) varied among species ($R^2_\beta$ = 0.69), decreased with understory cover ($R^2_\beta$ = 0.01) and increased with GLI ($R^2_\beta$ = 0.11; Fig. 5).

*Root traits*. – Root traits [i.e., specific root length (SRL) and root depth] varied only among species. These models explained 49% and 43% of the variation, with random effects accounting for an added 4% and 1%, respectively (Table 2).

*Variation explained by fixed effects*. – Fixed effects in the most parsimonious models explained between 35% ($\Delta^{15}N$) and 88% (leaf size) of the variability in all traits ($R^2_m$ in Table 2). Additional effects beyond those included in the most parsimonious models did not substantially increase the variation explained (Table 2). Apart from the models for iWUE and $\Delta^{15}N$, in which additional terms led to increases of 10 and 9%, none of the variables added in steps three through five did much to increase the variance explained beyond that explained by species alone (Table S3 in Supplementary Information). Moreover, small differences between $R^2_m$ and $R^2$ conditioned on random effects ($R^2_c$) suggest that only a small proportion of variation traits was due to unexplained variation among sites, experimental treatments and plots (Table 2, Table S.4 in Supplementary Information).

Finally, our analysis of semi-partial coefficients ($R^2_\beta$) showed that after accounting for other fixed effects in the model, the majority of variation (i.e., $R^2_\beta = 0.37$ to 0.88) in all traits is related to differences among species (Fig. 6). Following variation associated with species, variation in leaf traits is most strongly associated with GLI (SLA = 0.11, LNC = 0.06, $N_{area}$ = 0.29, leaf size = 0.27 and LDMC = 0.12). Understory cover was the only additional fixed effect in the model for plant height (explaining 19% of the variation after accounting for species). The model for SSD also included GLI [$R^2_\beta = 0.11$ accounting for species ($R^2_\beta = 0.69$) and understory cover ($R^2_\beta = 1\%$)]. Resource and environmental variables were relatively more important in explaining variation in isotope-derived whole plant traits. For iWUE, the order of importance of resources and environmental variables was GLI ($R^2_\beta = 0.36$) > soil N ($R^2_\beta = 0.05$) > understory cover ($R^2_\beta = 0.02$), while the order for $\Delta^{15}N$ was CMD ($R^2_\beta = 0.23$) > soil N ($R^2_\beta = 0.14$) > understory cover ($R^2_\beta = 0.2$).

## Discussion

Trait-based models of ecological communities are appealing because they promise greater generality, predictive power, and ability to scale between community and ecosystem levels of organization (McGill et al. 2006, Suding et al. 2008). These approaches commonly ignore trait variation within species, an assumption that has rarely been tested (Shipley et al. 2016). Our results highlight the importance of understanding the inference (sampling) scope when using single trait values to represent species in a plant community. Although interspecific variability exceeded intraspecific variability for most traits, levels of intraspecific variability were considerable. Within species, most traits varied significantly along multiple gradients (i.e., in light, understory cover, and/or soil N), but that variation did not lead to species cross-over. Thus, species rankings established in one set of environmental and resource conditions appear to hold under a broader range of conditions. In contrast, rankings may not be valid when individual species are sampled under different conditions (e.g., a shade-tolerant species sampled in the shade compared to an intolerant species sampled in a gap). Future studies examining the generality of our findings in other ecosystems, for other plant groups, and over larger gradients in resources and environmental conditions would be worthwhile. High levels of intraspecific variability suggest that a larger sample size may be required when characterizing reaction norms across a wider range of environmental conditions.

STABLE ISOTOPE DERIVED WHOLE PLANT TRAITS

The range of variation in foliar N stable isotope discrimination relative to soil ($\Delta^{15}N$) provides a metric of niche-partitioning in N use strategies among plants due to differences in the forms, timing and depth of N uptake from soil, as well as internal plant N distribution and plant-mycorrhizal associations (e.g., Nadelhoffer et al. 1996, Gubsch et al. 2011). Estimates of $\Delta^{15}N$ also control for foliar $\delta^{15}N$ tracking of soil $\delta^{15}N$ across sites, which can otherwise obscure or confound differences in plant uptake strategies (Houlton et al. 2007). We observed significant variation in $\Delta^{15}N$ that confirms high intraspecific variation in N use strategies for the understory species examined. Nearly all species displayed negative $\Delta^{15}N$ values, which is typical of plants that rely on soil inorganic N (Nadelhoffer et al. 1996). For these species, the decrease in $\Delta^{15}N$ (i.e., indicating a broader span between plant and soil $\delta^{15}N$) with increasing understory cover indicates intensified niche partitioning and diminished variation in niche-breadth (e.g., timing, depth, and form) of N uptake when plant competition for N is high (Gubsch et al. 2011). However, the low semi-partial coefficient ($R^2_{\beta}$) for understory cover (0.02) suggests effects of competition on $\Delta^{15}N$ are relatively weak. Interspecific differences (i.e., phylogenetic N use strategies) and broader scale variation in abiotic variables (soil N and climatic moisture deficit) were relatively more important. Low $\Delta^{15}N$ values (i.e, broader span between plant and soil $\delta^{15}N$) in most species were also associated with low soil N, consistent with patterns of overstory $\delta^{15}N$ in these forests (Perakis et al. 2011, 2015). This can reflect greater N niche partitioning at low N sites, increased reliance on $NH_4^+$ uptake, and/or greater $\delta^{15}N$ discrimation by mycorrhizae (Gubsch et al. 2011). A majority of species also displayed increasing $\Delta^{15}N$ values (i.e., narrower span between plant and soil $\delta^{15}N$) with higher moisture deficits, consistent with greater uptake of N from deeper soil horizons (Nadelhoffer et al. 1996) and/or uptake of soil nitrate that has been isotopically enriched by denitrification (Austin & Vitousek 1998). At the level of individual species, the unusually high $\delta^{15}N$ (i.e., the only species with positive values of $\Delta^{15}N$) and rather ordinary $\delta^{13}C$ values of *Chimaphila menziesii* is noteworthy. Species in this genus can be myco-heterotrophic, and our stable isotope patterns suggest it derived most N directly from fungal partners while deriving C from either photosynthesis or recently produced plant photosynthates (Zimmer et al. 2007).

Summer moisture deficits are generally believed to limit photosynthesis and stomatal conductance in the Pacific Northwest (e.g., Waring & Franklin 1979). Our results showed that iWUE of understory species increased, rather than decreased, with GLI. This suggests increases in photosynthetic carbon assimilation relative to stomatal conductance at low overstory densities are due to resource levels and/or increasing stomatal conductance associated with higher relative humidity in closed canopies. A positive correlation between iWUE and leaf nitrogen per unit area ($N_{area;}$ $r = 0.38$, $p<0.0001$), which also increases with GLI (Fig. 4), indicates that greater assimilation rates with lower overstory densities was the more likely mechanism. The negative relationship between iWUE and understory cover may reflect effects of understory cover on light below the 1 m camera height that is not captured by GLI. However, in contrast to previous work, which focused on overstory trees (e.g., Waring & Franklin 1979) our results suggest a dominant role of light in determining iWUE of understory species and that the role of water limitations in forests in the Pacific Northwest may vary for different vegetation layers.

## LEAF TRAITS

The relationships between leaf traits and resources, environmental conditions and understory cover may have resulted from a trade-off between high structural investments per unit area in well-lit conditions versus in light absorption in shady conditions. This interpretation is supported by the negative relationship of SLA and leaf size to GLI, and the positive of LDMC to GLI (Fig. 4). This is consistent with the reversed response of SLA and LDMC (i.e., positive) in response to understory cover (Fig. S2). Decreases in SLA with irradiance is associated with a lower density of thylakoids per stroma volume/grana and increases in palisade parenchyma cell thickness, chloroplasts and nitrogen per unit area ($N_{area}$), biochemical photosynthetic capacity and respiration (Givnish 1988, Lambers, Chapin & Pons 1998). In contrast, higher SLA in shade is associated with increased mass-based leaf nitrogen content and an increased proportion of spongy mesophyll leading to more efficient light capture per unit biomass and longer leaf lifespans. Longer leaf lifespans without additional structural investment are favored in forest understories characterized by relatively low wind speeds and high relative humidity (Westoby et al. 2002, Lusk et al. 2008). Lower mass-based, and higher area-based, LNC in sun relative to shade, respectively, have been documented for a wide variety of plants (Chazdon 1992, Ellsworth & Reich 1992, 1993, Givnish et al. 2004). Deciduous and evergreen species show similar plastic responses of SLA (or LMA) to shade as found in our study, likely as a result of selection for low construction and maintenance costs (Lusk et al. 2008).

STEM TRAITS

The observed relationships of stem traits to light transmission and understory cover may be related to trade-offs between competition in dense understories and mechanical safety where canopy conditions are more open (Givnish 1995). Stem specific density (SSD) increased with GLI and decreased with understory cover, whereas height increased (Fig. 4). Shade and competition with neighbors can decrease mechanical stability as a result of lower stem diameter relative to height, reducing SSD and root:shoot ratios. However, plants can increase SSD, and root:shoot ratios after overstory removal and decrease leaf area to control stem deflection in wind (Henry & Thomas 2002, Briggs et al. 2012). Allocation of a greater proportion of a plant's resources to stems in the form of height or SSD comes at a cost of allocation to photosynthetically-active leaves. Our results empirically support the theoretical prediction that the optimal allocation to stems increases with competition (i.e., understory cover) and productivity (Givnish 1982). Additional effects of soil N did not improve models of SSD and height, suggesting productivity effects can be predicted locally with understory cover and light transmission. Alternatively, local variation in soil total N is not clearly reflective of plant-available N. High SSD can lead to lower hydraulic conductance (Meinzer et al. 2008, Chave et al. 2009). However, the benefits of increasing SSD for mechanical safety at high GLI and low understory cover appear to outweigh the potential costs to hydraulic conductance.

## ROOT TRAITS

Variation in specific root length (SRL) and rooting depth was not strongly related to resource or environmental variables assessed here. Root development patterns may be similar to those for leaves, where longer root investments per unit mass can increase the capacity for uptake and proliferation at the expense of tolerance for xeric or infertile soils (Fitter 1985, Eissenstat 1991, Eissenstat & Yanai 1997). As such, positive relationships between SRL and resources, especially, soil N and moisture, and negative relationships with competition may be expected. On the other hand, negative relationships between SRL and soil resources, and positive relationships between SRL and competition support the hypothesis that plant allocation to roots is lower where resources are high, and necessarily higher where resources are low (Agren &

Franklin 2003, Ostonen et al. 2007). The lack of clear support for either alternative in our data might suggest that SRL generally does not vary in response to resources levels, at least within the resource range measured in our study. It is possible, however, that relevant resources were not measured at an appropriately fine scale. For example, soil properties may vary significantly within the sampling radius of our plots as a result of variation in tree and understory species composition, pit and mound topography and soil disturbance (e.g., Ettema & Wardel 2002). Moreover, understory species vary with respect to root branching patterns and the presence of fine roots. We focused on the outermost live roots, including only fine roots when possible. For species lacking fine roots (e.g., *Goodyera oblongifolia*, *Disporum* spp., *Asarum caudatum*), we measured the finest of the live coarse roots. Controlling for variation among root branching orders would have allowed us to better detect such relationships, but was not possible in our study (Pregitzer et al. 2002, Ostonen et al. 2007). Similar interpretations may apply to the results for maximum rooting depth (Antos and Halpern 1997).

## Author's Contributions

JIB, KJP and SSP conceived the ideas and designed the methodology; CEL, SCM and JIB collected the data; JIB analyzed the data; JIB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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## Data Accessibility

Data will be deposited in the Dryad Digital Repository: https://datadryad.org/resource/doi:10.5061/dryad.8125b

## Supporting Information

**Fig. S1.** Illustration of species cross-over.

**Appendix S1.** Detailed information related to the study area, data collection and statistical analysis.

**Table S1.** Step-wise model selection process.

**Table S2.** Number of individuals sampled by species and trait.

**Table S3.** Alternative model comparisons.

**Table S4.** Correlations among fixed effects.

**Fig. S2.** Relationship between leaf traits and understory cover.

**Fig. S3.** Relationship of leaf N per area to soil N.

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Table 1. Leaf, stem and root traits examined and their hypothesized reaction norms (positive or negative) along gradient in resources and environmental conditions relevant to understory plant species.

| | Gradients in resources and environmental conditions | | | |
|---|---|---|---|---|
| Trait | GLI | Understory cover | Soil nitrogen | CMD |
| *Whole plant traits* | | | | |
| $\Delta^{15}N$ | + | - | + | - |
| iWUE | + | - | + | - |
| *Leaf traits* | | | | |
| SLA | - | + | + | - |
| LNC | - | + | + | - |
| $N_{area}$ | + | - | + | - |
| Leaf size | - | + | + | - |
| LDMC | + | - | - | + |
| *Stem traits* | | | | |
| Plant height | + | + | + | - |
| SSD | - | + | - | + |
| *Root traits* | | | | |
| SRL | + | - | + | - |
| Rooting depth | - | + | - | + |

Abbreviations: GLI = gap light index, CMD = climatic moisture deficit, $\Delta^{15}N$ = nitrogen stable isotope discrimination, iWUE = intrinsic water use efficiency, SLA = specific leaf area, LNC = leaf nitrogen content, $N_{area}$ = leaf nitrogen per unit area, LDMC = leaf dry matter content, SSD = stem specific density, SRL = specific root length.

Table 2. Comparisons of ΔAICc, AIC weights (*w*), and variability explained by fixed effects ($R^2_m$) and fixed and random effects (*k* = number of parameters) combined ($R^2_c$) among three top models selected using multistep modeling processes (Table S1). The selected model is shown in bold text. Results from all model comparisons are provided in Table S3.

| Model by dependent variable | *k* | ΔAICc | *w* | $R^2_m$ | $R^2_c$ |
|---|---|---|---|---|---|
| WHOLE PLANT TRAITS | | | | | |
| *$\Delta^{15}N$* | | | | | |
| **Species - $Cover_U$ + $N_{soil}$ + CMD** | **63** | **0.00** | **0.85** | **0.35** | **0.47** |
| Species - $Cover_U$ + $N_{soil}$ + CMD - CMD*$N_{soil}$ + CMD*$Cover_U$ | 65 | 3.74 | 0.13 | 0.35 | 0.47 |
| Species + $Cover_U$ + $N_{soil}$ - $N_{soil}$*$Cover_U$ | 64 | 8.59 | 0.01 | 0.32 | 0.44 |
| *iWUE* | | | | | |
| Species - $Cover_U$ + GLI - $N_{soil}$ + $N_{soil}$*$Cover_U$ | 64 | 0.00 | 0.25 | 0.49 | 0.54 |
| Species - $Cover_U$ + GLI - $N_{soil}$ | 63 | 0.01 | 0.24 | 0.49 | 0.54 |
| **Species - $Cover_U$ + GLI** | **62** | **0.96** | **0.15** | **0.49** | **0.53** |
| LEAF TRAITS | | | | | |
| *Log(specific leaf area)* | | | | | |
| Species - $Cover_U$ + GLI + $Cover_U$*GLI + $N_{soil}$ | 64 | 0.00 | 0.36 | 0.80 | 0.81 |
| **Species - $Cover_U$ - GLI + $Cover_U$*GLI** | **63** | **1.14** | **0.20** | **0.80** | **0.80** |
| Species + $Cover_U$ - GLI + $Cover_U$*GLI + $N_{soil}$ + $N_{soil}$*GLI - $N_{soil}$*$Cover_U$ | 66 | 1.72 | 0.15 | 0.80 | 0.80 |
| *Log(leaf nitrogen content)* | | | | | |
| **Species - $Cover_U$ - GLI + $Cover_U$*GLI** | **64** | **0.00** | **0.37** | **0.49** | **0.55** |
| Species - $Cover_U$ - GLI + $Cover_U$*GLI - CMD | 65 | 2.21 | 0.12 | 0.53 | 0.57 |

| | | | | | |
|---|---|---|---|---|---|
| Species - $Cover_U$ - GLI + $Cover_U$*GLI + $N_{soil}$ | 65 | 2.30 | 0.12 | 0.55 | 0.59 |
| *Log(leaf nitrogen per unit area)* | | | | | |
| **Species + GLI - $N_{soil}$** | **63** | **0.00** | **0.37** | **0.66** | **0.66** |
| Species + GLI - $N_{soil}$ - CMD | 64 | 0.90 | 0.23 | 0.66 | 0.66 |
| Species + GLI + $N_{soil}$ – GLI*$N_{soil}$ | 64 | 2.10 | 0.13 | 0.66 | 0.66 |
| *Log(Leaf size)* | | | | | |
| **Species + $Cover_U$ - GLI** | **62** | **0.00** | **0.25** | **0.87** | **0.88** |
| Species + $Cover_U$ - GLI + $Cover_U$*GLI | 63 | 0.00 | 0.25 | 0.88 | 0.88 |
| Species + $Cover_U$ - GLI + $N_{soil}$ | 63 | 0.30 | 0.22 | 0.88 | 0.88 |
| *Log(LMDC)* | | | | | |
| **Species + $Cover_U$ + GLI - $Cover_U$*GLI** | **64** | **0.00** | **0.41** | **0.80** | **0.81** |
| Species + $Cover_U$ + GLI - $Cover_U$*GLI - CMD | 65 | 0.32 | 0.35 | 0.80 | 0.81 |
| Species + $Cover_U$ + GLI - $Cover_U$*GLI - $N_{soil}$ | 65 | 1.57 | 0.19 | 0.80 | 0.81 |
| STEM TRAITS | | | | | |
| *Log(height)* | | | | | |
| Species + $Cover_U$ + $N_{soil}$ | 62 | 0.00 | 0.21 | 0.78 | 0.78 |
| Species + $Cover_U$ - GLI | 63 | 0.12 | 0.20 | 0.78 | 0.78 |
| **Species + $Cover_U$** | **62** | **0.18** | **0.19** | **0.78** | **0.78** |
| *Log(stem specific density)* | | | | | |
| **Species + $Cover_U$ + GLI - $Cover_U$*GLI** | **51** | **0.00** | **0.40** | **0.71** | **0.71** |
| Species + $Cover_U$ + GLI - $Cover_U$*GLI - CMD | 52 | 2.24 | 0.13 | 0.71 | 0.71 |
| Species - $Cover_U$ + GLI | 50 | 2.46 | 0.12 | 0.70 | 0.71 |
| ROOT TRAITS | | | | | |
| *Log(specific root length)* | | | | | |
| **Species** | **61** | **0.00** | **0.36** | **0.49** | **0.53** |

| | | | | | |
|---|---|---|---|---|---|
| Species + $Cover_U$ | 62 | 2.32 | 0.11 | 0.49 | 0.53 |
| Species - GLI | 62 | 2.33 | 0.11 | 0.49 | 0.53 |
| *Log(Root depth)* | | | | | |
| **Species** | **51** | **0.72** | **0.17** | **0.41** | **0.42** |
| Species - CoverU | 52 | 0.53 | 0.19 | 0.41 | 0.42 |
| Species - CoverU + GLI | 53 | 0.00 | 0.25 | 0.41 | 0.42 |

Abbreviations: $Cover_u$ = understory cover, $N_{soil}$ = soil nitrogen. See Table 1 for others.

Figure 1. Sources of variation in whole plant, leaf, stem and root traits. Relative variance decomposition at the species and within-species levels. Intraspecific variation was partitioned among and within sites.

Figure 2. Relationships of foliar stable isotope discrimination ($\Delta^{15}N$) to soil N (a-c), understory cover (d-f) and climatic moisture deficit (g-i). Ferns and graminoids are shown in the first column (a,d,g), forbs in the second (b,e,h), and shrubs in the third (c,f,i). Lines show model estimates from selected model (Table 2) for individual species plotted across the range of conditions in which they were sampled. Note differences in scale range for shrubs (c,f,i).

Figure 3. Relationships of intrinsic water use efficiency (iWUE) to gap light index (a-c), understory cover (d-f), and soil N (g-i). Ferns and graminoids are shown in the first column (a,d,g), forbs in the second (b,e,h), and shrubs in the third (c,f,i). Lines show model estimates from selected models (Table 2) for individual species plotted across the range of conditions in which they were sampled (see Fig. 2 for legend).

Figure 4. Relationships between leaf traits (rows) and gap light index (GLI). Ferns and graminoids are shown in the first column (a,d,g,j,m), forbs in the second (b,e,h,k,n), and shrubs in the third (c,f,i,l,o). Lines show model estimates from selected models (Table 2) for individual species plotted across the range of conditions in which they were sampled (see Fig. 2 for legend). Understory cover was held at “low” levels (20%, rather than species means) for plotting trait – GLI relationships when final models included an interaction between GLI and understory cover (SLA and LDMC) because relationships between traits and GLI were less pronounced at high

understory cover. Similarly, in these cases GLI was held constant at high levels (0.40) for plotting trait-understory cover relationships (Fig. S.3.1). Relationships are plotted on the log scale (y-axis) to ease interpretation. Note differences in scale range for height between (b), and (a) and (c).

Figure 5. Relationship between (a) plant height and understory cover and (b) stem specific density (SSD) and gap light index at low understory cover. Lines show model estimates from selected models (Table 2) for individual species plotted across the range of conditions in which they were sampled (see Fig. 2 for legend). Relationships are plotted on the log scale (y-axis) to ease interpretation.

Figure 6. Proportion of the total variance explained by fixed effects in the selected models for each whole plant, leaf, stem and root trait.

Figure 1.

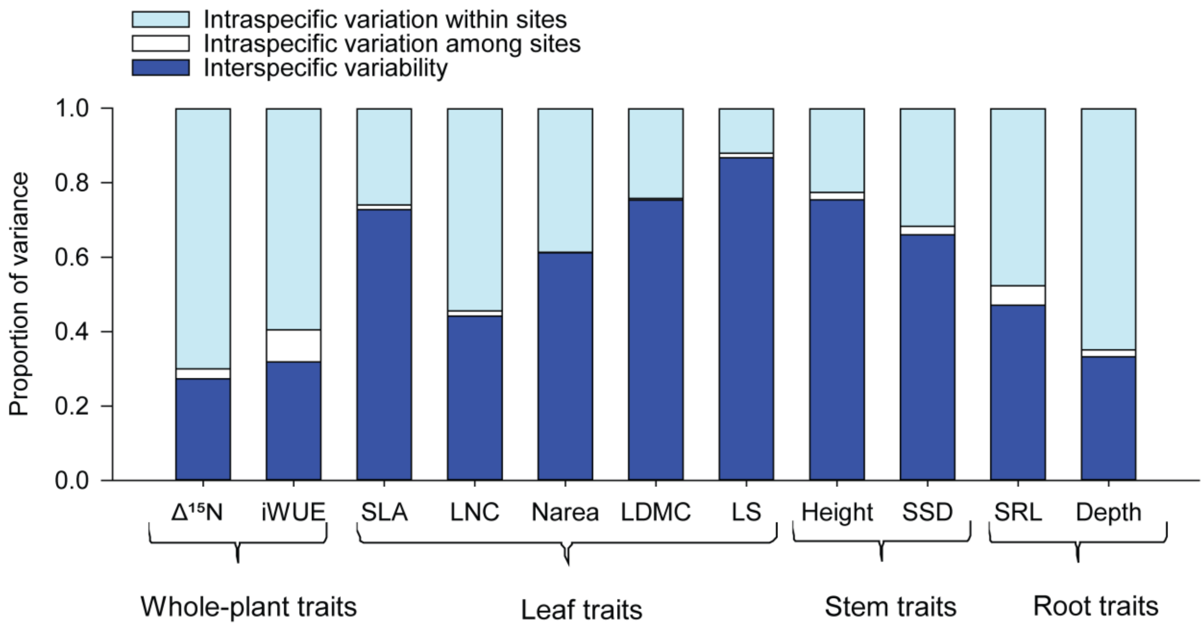
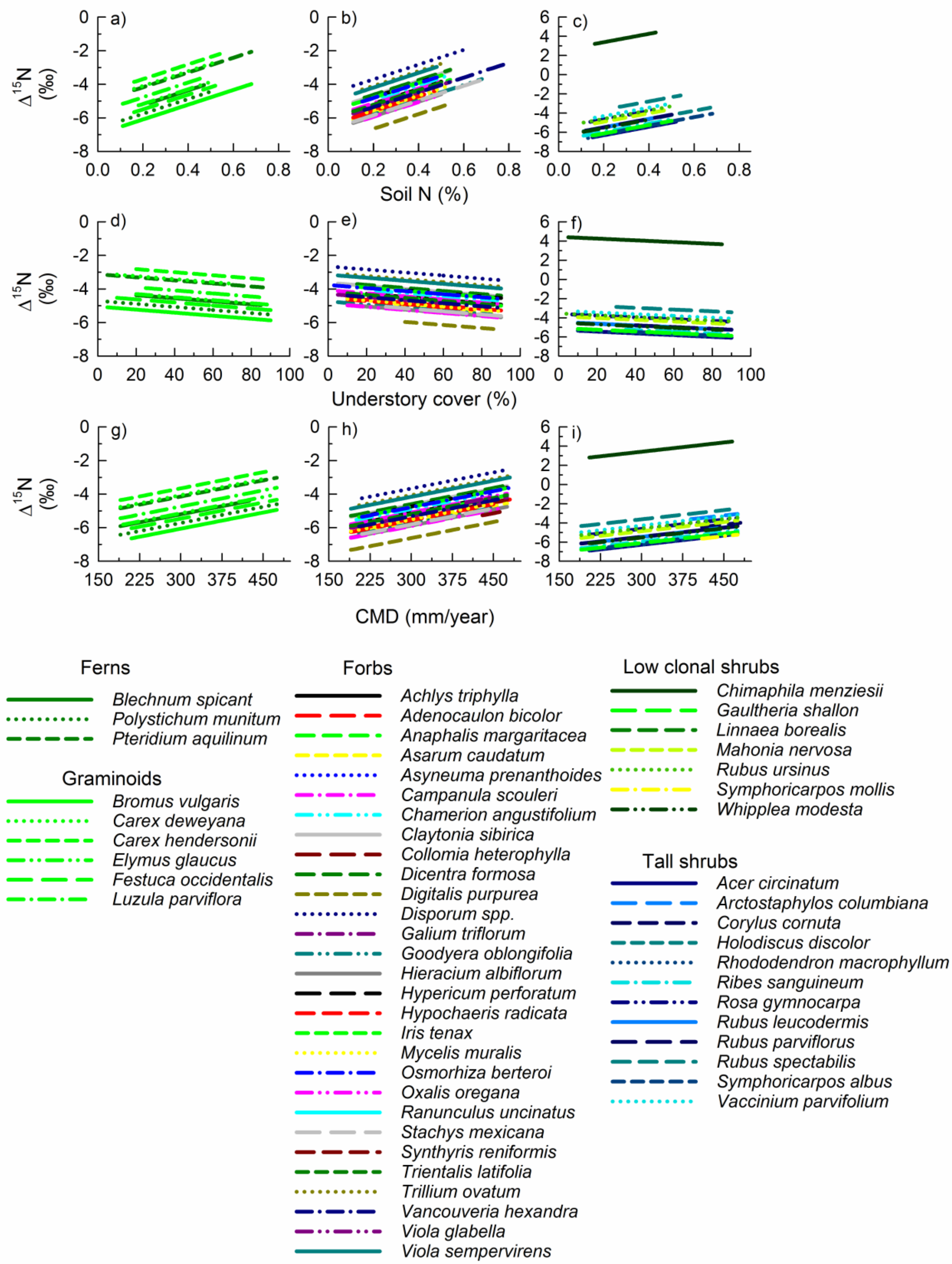

Intraspecific variation within sites
Intraspecific variation among sites
Interspecific variability
Proportion of variance
1.0
0.8
0.6
0.4
0.2
0.0
Δ¹⁵N
iWUE
SLA
LNC
Narea
LDMC
LS
Height
SSD
SRL
Depth
Whole-plant traits
Leaf traits
Stem traits
Root traits

Figure 2.



a)
b)
c)
d)
e)
f)
g)
h)
i)
$\Delta^{15}$N (‰)
Soil N (%)
Understory cover (%)
CMD (mm/year)
Ferns
Blechnum spicant
Polystichum munitum
Pteridium aquilinum
Graminoids
Bromus vulgaris
Carex deweyana
Carex hendersonii
Elymus glaucus
Festuca occidentalis
Luzula parviflora
Forbs
Achlys triphylla
Adenocaulon bicolor
Anaphalis margaritacea
Asarum caudatum
Asyneuma prenanthoides
Campanula scouleri
Chamerion angustifolium
Claytonia sibirica
Collomia heterophylla
Dicentra formosa
Digitalis purpurea
Disporum spp.
Galium triflorum
Goodyera oblongifolia
Hieracium albiflorum
Hypericum perforatum
Hypochaeris radicata
Iris tenax
Mycelis muralis
Osmorhiza berteroi
Oxalis oregana
Ranunculus uncinatus
Stachys mexicana
Synthyris reniformis
Trientalis latifolia
Trillium ovatum
Vancouveria hexandra
Viola glabella
Viola sempervirens
Low clonal shrubs
Chimaphila menziesii
Gaultheria shallon
Linnaea borealis
Mahonia nervosa
Rubus ursinus
Symphoricarpos mollis
Whipplea modesta
Tall shrubs
Acer circinatum
Arctostaphylos columbiana
Corylus cornuta
Holodiscus discolor
Rhododendron macrophyllum
Ribes sanguineum
Rosa gymnocarpa
Rubus leucodermis
Rubus parviflorus
Rubus spectabilis
Symphoricarpos albus
Vaccinium parvifolium

Figure 3.

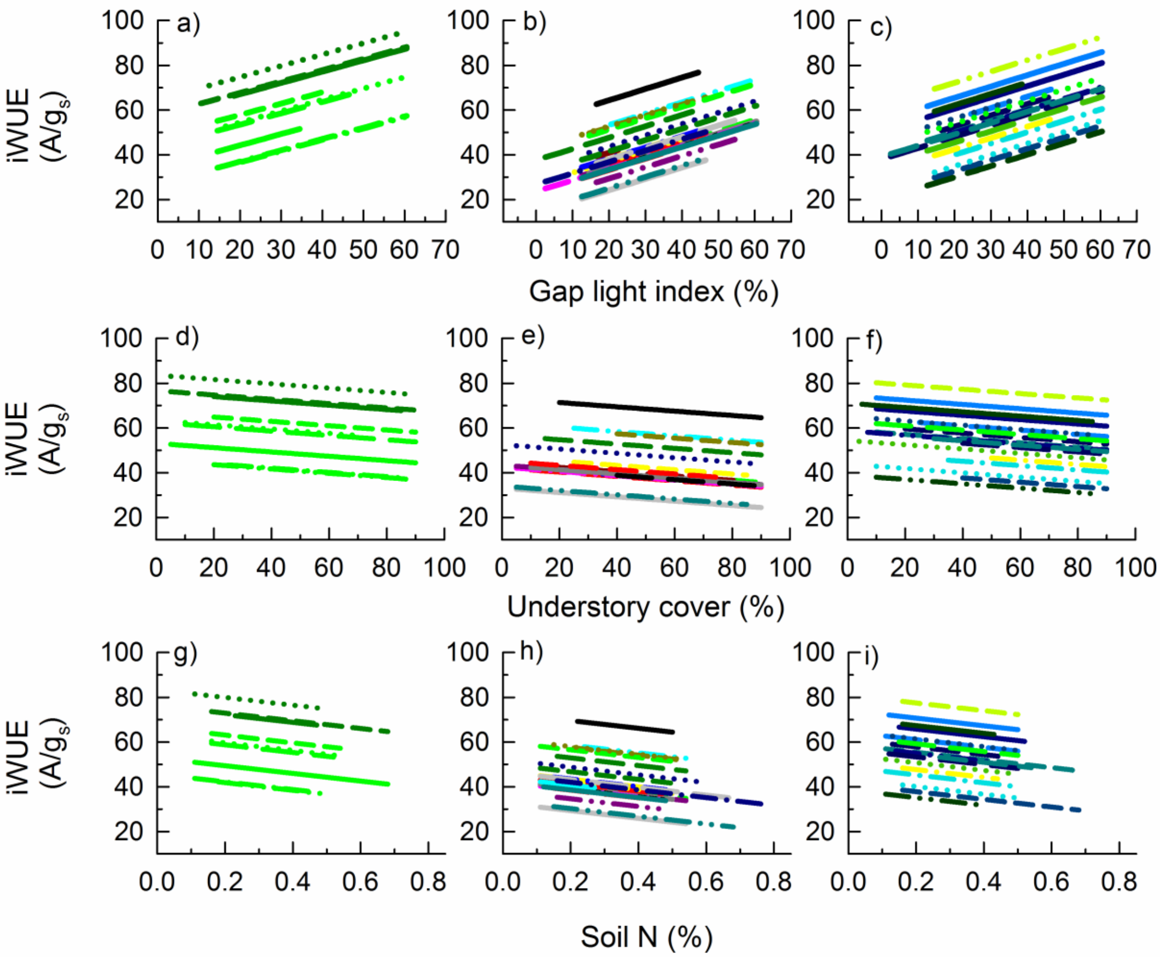


Figure 4.

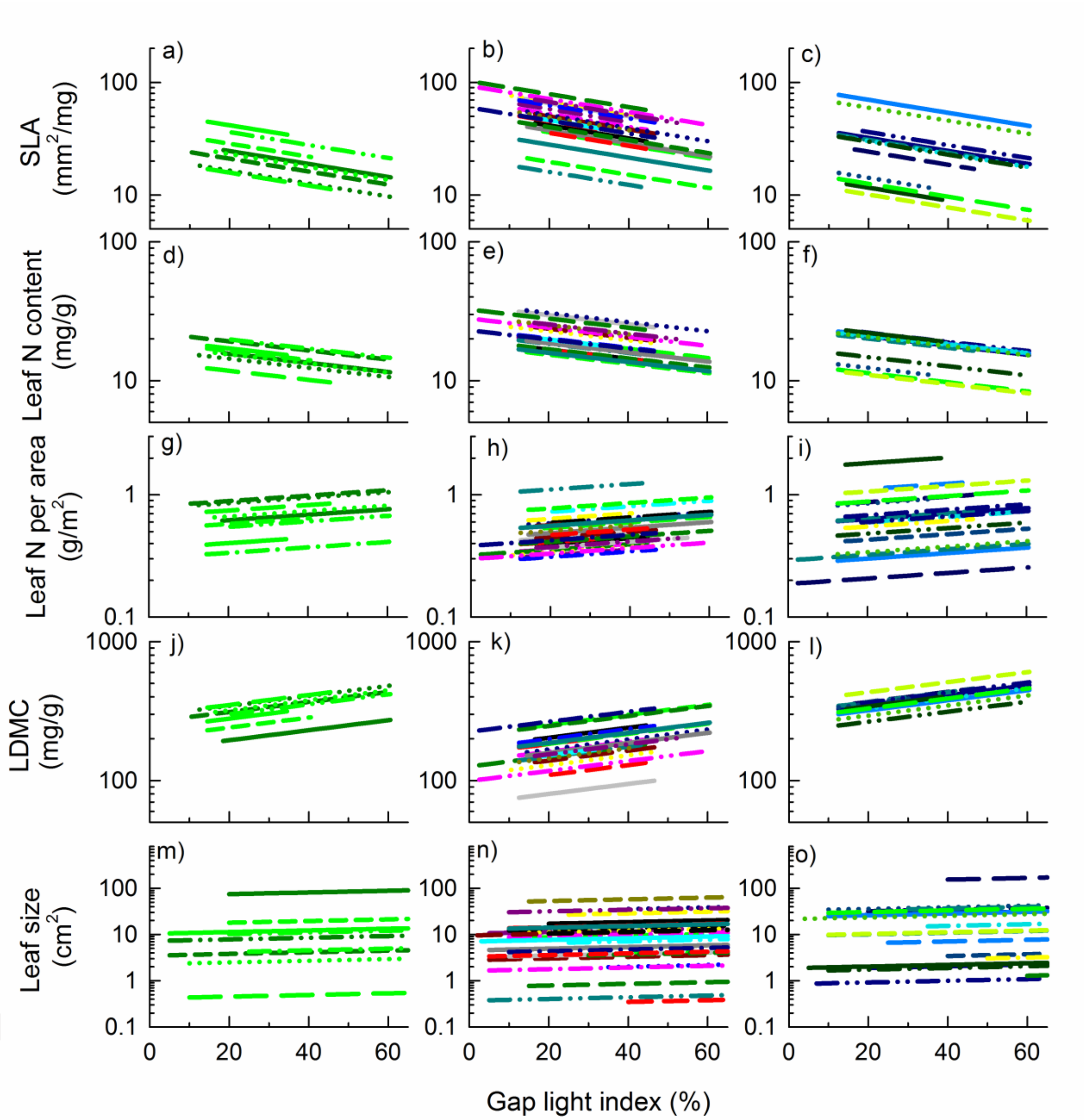

a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
k)
l)
m)
n)
o)
SLA (mm$^2$/mg)
Leaf N content (mg/g)
Leaf N per area (g/m$^2$)
LDMC (mg/g)
Leaf size (cm$^2$)
Gap light index (%)
0
20
40
60
0.1
1
10
100
1000

Figure 5.

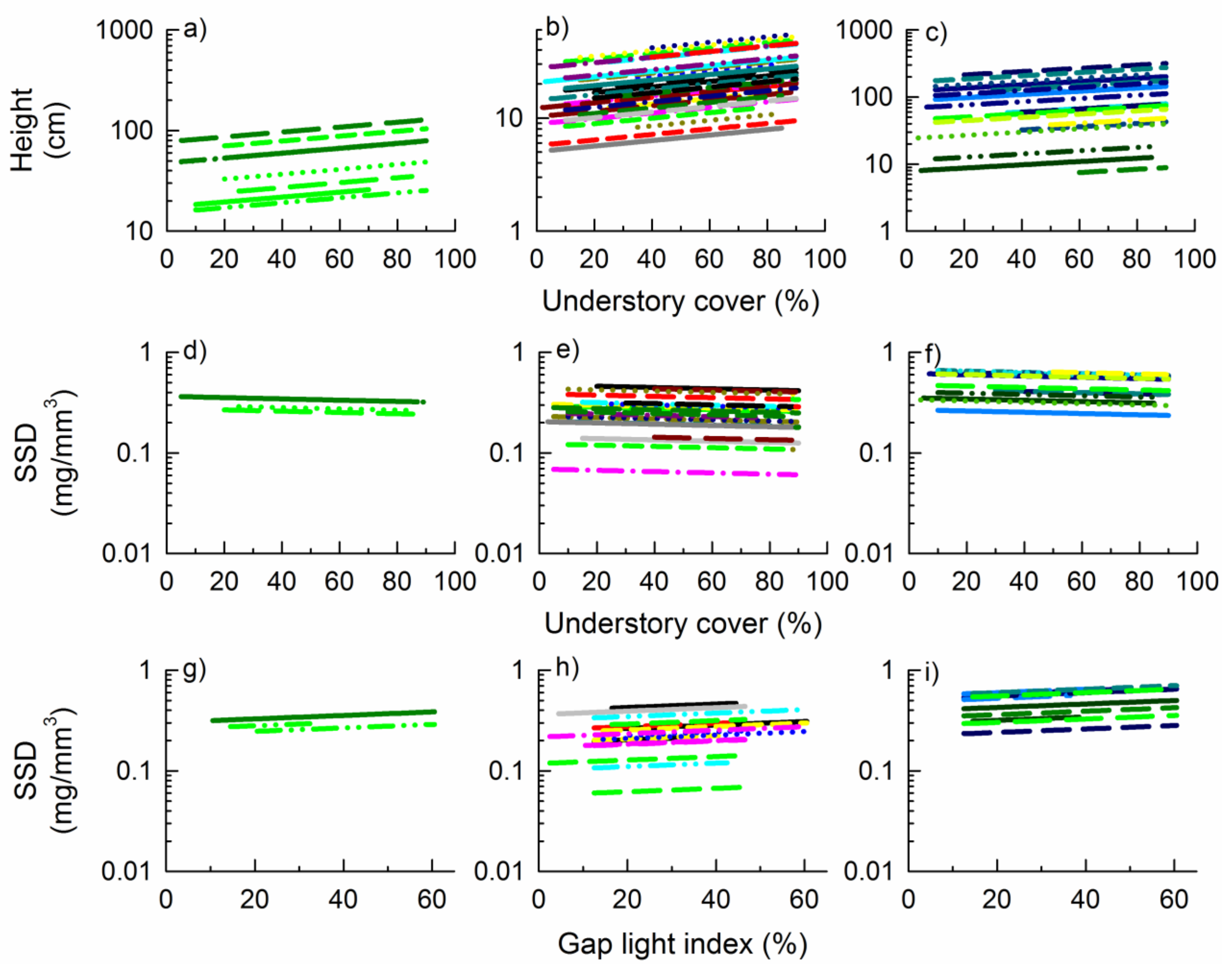
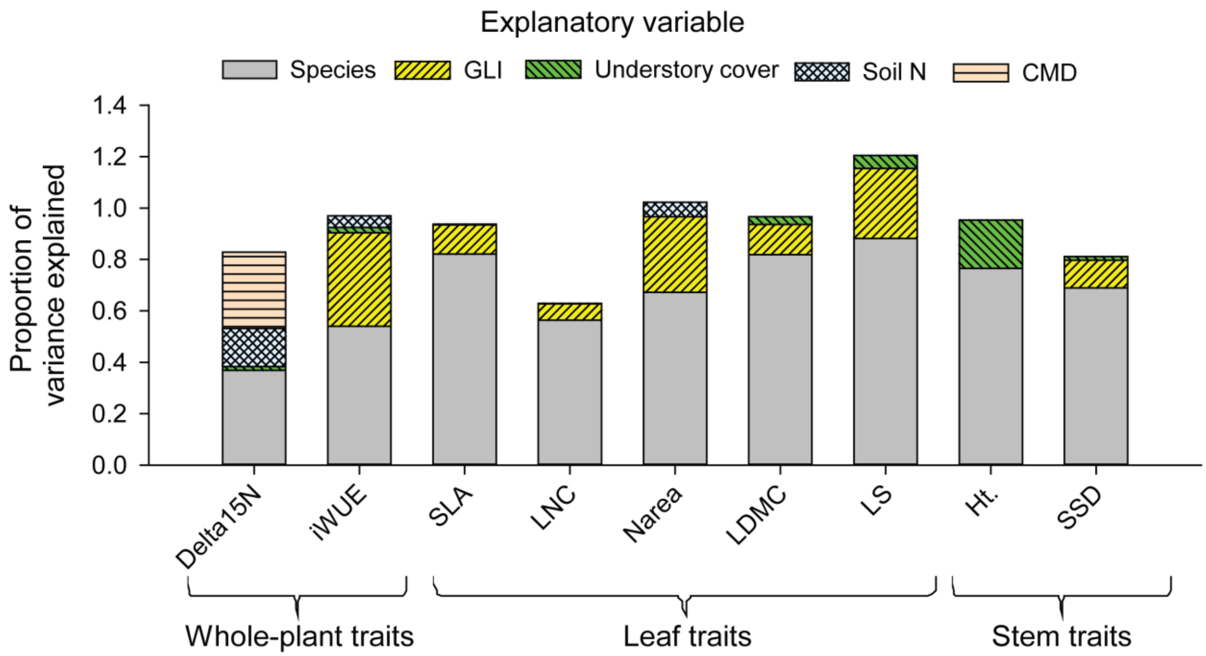

a)
b)
c)
Height (cm)
1000
100
10
1
0
20
40
60
80
100
Understory cover (%)
d)
e)
f)
SSD (mg/mm3)
1
0.1
0.01
g)
h)
i)
Gap light index (%)

Figure 6.



Explanatory variable
Species
GLI
Understory cover
Soil N
CMD
Proportion of variance explained
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Delta15N
iWUE
SLA
LNC
Narea
LDMC
LS
Ht.
SSD
Whole-plant traits
Leaf traits
Stem traits