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Regular article

Title: Integrating nitrogen fixing structures into above- and belowground functional trait spectra in soy (*Glycine max*).

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Running Header: Multiple dimensions of intraspecific trait variation in crops.

Keywords

Functional trait; *Glycine max*; intraspecific trait variation; Leaf Economics Spectrum; nitrogen fixation; nodules; Root Economics Spectrum; root traits.

Abstract

- **Aims:** Phenotypic trait variation across environmental gradients and through plant ontogeny is critical in driving ecological processes, especially in agroecosystems where single genotypes exist in high abundances. While variability in root traits plays a key role in belowground processes, few studies have identified the presence of an intraspecific “Root Economics Spectrum” (RES) within domesticated plants. Furthermore, little is known regarding if an intraspecific RES changes through plant ontogeny, and how tropic interactions – namely root nodulation – relate to above- or belowground trait spectra.
- **Methods:** We evaluated covariation among 12 root, nodule, leaf, and stem traits in 134 plants of a single genotype of soy (*Glycine max*). Variation in these traits was assessed across five managed environmental conditions, and three plant ontogenetic stages.
- **Results:** Root traits covaried along an intraspecific RES that represents a trade-off between resource acquisition and resource conservation. Variation along the RES was closely coordinated with hydraulic traits, but was orthogonal to nodule and leaf economics traits. Trait relationships varied strongly across managed environmental conditions and plant developmental stages.
- **Conclusions:** Our results indicate the presence of an intraspecific RES in soy that is independent of root nodule investment. Patterns of phenotypic variation in below and aboveground soy traits demonstrate multivariate trait syndromes vary across environmental gradients and are dynamic through plant ontogeny.

Introduction

Understanding the dimensions of functional trait variation among plant species has become a major unifying theme in terrestrial ecology (Diaz et al. 2016; Westoby 1998; Westoby et al. 2002; Wright et al. 2004), with explicit implications for understanding differences in realized plant performance (e.g. Poorter and Bongers 2006), plant species coexistence (e.g. Kraft et al. 2015), and rates of ecosystem functioning (e.g. Cadotte et al. 2009; Cornwell et al. 2008; Lavorel and Garnier 2002). In recent years this area of ecological enquiry has expanded beyond the central goal of evaluating trait variation among plant species in “natural” ecosystems, into two emerging realms: i) understanding the extent, causes, and consequences of genotypic and phenotypic intraspecific variation in traits (Albert et al. 2010a; Albert et al. 2010b; Messier et al. 2010; Violle et al. 2012), and ii) applying trait-based principles into the sustainable management of plant-based ecosystems (Damour et al. 2018; Garnier and Navas 2012; Martin and Isaac 2015; 2018).

Evaluating intraspecific trait variation in crops has emerged as a novel application of trait-based ecology that addresses both of these avenues. Evidence from a range of crops including wheat, maize, soy, sunflower, and coffee, indicate that key dimensions of trait variability among wild plants – namely leaf trait co-variation across the Leaf Economics Spectrum (LES) (Wright et al. 2004) – also describe trait variation within and among domesticated plants (Gagliardi et al. 2015; Martin et al. 2017; Mason and Donovan 2015; Mason et al. 2013). These studies further indicate that evaluating variation in crop leaf traits refines our understanding of i) the ecological consequences of plant domestication (Milla et al. 2014; Milla et al. 2015), and ii) the plant traits that govern rates of key agroecosystem functions such as yield (Hayes et al. 2018) and nutrient cycling (Garcia-Palacios et al. 2013).

To date the majority of research on trait variation in crops has focused on “domestication traits” associated with yield, or LES and other aboveground traits (Gagliardi et al. 2015; Martin et al. 2018; Martin et al. 2017; Mason and Donovan 2015; Mason et al. 2013). In comparison, beyond a few exceptions (Isaac et al. 2017; Milla et al. 2014; Roucou et al. 2018) there are a limited number of studies evaluating if, or how, plants of the same genotype vary across other functional trait spectra. Specifically, analogous to the LES, hypotheses suggest that plants differentiate along a “Root

Economics Spectrum” (RES), which is linked with plant responses to belowground resource availability and uptake (Bardgett et al. 2014; Ma et al. 2018; Prieto et al. 2015; Roumet et al. 2016). While the traits forming, and dimensions of, a RES continue to be refined (de la Riva et al. 2018; Laliberte 2017; Ma et al. 2018) certain species-comparative studies indicate that high values of specific root length (SRL), specific root area (SRA), and frequency of root tips (e.g. specific root tip density (SRTD; tips mg^{-1})), coupled with small average root diameters (RD) describe a resource-acquisitive ecological strategy; the opposite suite of traits reflects a resource-conservative ecological strategy (Kong et al. 2014; Mommer and Weemstra 2012; Prieto et al. 2015; Roumet et al. 2016; Weemstra et al. 2016; Zadworny et al. 2017).

Two studies have indicated the presence of an intraspecific RES, owing to phenotypic plasticity in roots across environmental gradients (Hajek et al. 2013; Isaac et al. 2017). But these patterns observed in woody perennials may not be generalizable across all crops. For instance, soybean (*Glycine max* (Fabaceae); the focus of our study here) is the world’s fourth-most widespread crop species, and fixes atmospheric dinitrogen (N_2) vis-à-vis symbiotic N_2 -fixing *Rhizobium* bacteria housed within root nodules. Extensive experimental studies on soy suggest that the dry mass or counts of root nodules are positively associated with soil moisture (e.g. Djekoun and Planchon 1991; Nasielski et al. 2015) and negatively associated with soil N availability (e.g. Isaac et al. 2014; Salvagiotti et al. 2008; Salvagiotti et al. 2009; Streeter 1985). By extension one may hypothesize that investment in nodules (in terms of nodule mass and counts) covaries along an intraspecific RES. However, direct tests of prospective integration of belowground trophic interactions directly along an intraspecific RES, or other trait dimensions, remain limited in the functional trait literature (Laliberte 2017; but see recent analyses of mycorrhizal colonization by Ma et al. 2018; McCormack et al. 2015).

In agroecosystems, management regimes that actively manipulate above- and belowground environmental conditions are likely to influence the expression of, and relationships among, crop traits. Agroforestry systems in particular result in strong gradients of both above- and belowground abiotic conditions; when employed in soy cultivation, these gradients have been linked with microclimatic modifications that result in stability of yield and N_2 -fixation under environmental stress (Nasielski et al. 2015).

More specifically, crops cultivated in greater proximity to perennial trees (i.e. within < 2 m of tree stems) generally experience lower photosynthetically active radiation levels, evapotranspiration rates, plant- and soil water deficits, and temperatures, as compared to areas further from trees, or those grown in monoculture (Reynolds et al. 2007). In soy-based agroforestry systems, these environmental gradients have been found to result in the expression of more “resource acquiring” leaf traits with proximity to trees (Hayes et al. 2018). At the same time, heightened belowground competition for nutrients and water in closer proximity to trees would be hypothesized to result in crops within agroforestry arrangements expressing more “resource acquiring” RES traits. Alternatively, increases in nodule traits would likely be observed in response to increasing light availability in monoculture (Nasielski et al. 2015) partially in response to increased aboveground plant growth under high light (Voisin et al. 2010; Whitehead and Isaac 2012) and/or other environmental stimuli.

Another outstanding uncertainty is how relationships among intraspecific leaf-, root-, or other trait spectra change across plant ontogeny and/ or environmental conditions, and how these changes may reflect shifts in plant functional biology through different developmental stages. For instance, multiple studies have demonstrated wide variation in LES traits occurring through plant ontogeny, with implications for understanding how plant resource allocation changes over the course of reproductive onset (Mason and Donovan 2015; Mason et al. 2013; Sendall and Reich 2013; Thomas 2010; Hayes et al. 2018). But comparatively, to our knowledge, there are no studies that have assessed i) ontogenetic patterns in RES trait variation, or ii) if relationships between different suites of above- and belowground traits shift through plant ontogeny. For soy, these ontogenetic patterns may be particularly distinct across the stages of pod development (Fehr et al. 1971).

Our analysis was designed to address these uncertainties, by assessing the following questions surrounding phenotypic trait variation of the world’s most widely cultivated N-fixing plant species: 1) is there an intraspecific RES in soy, and if so 2) does N₂-fixing nodule mass and count co-vary along an intraspecific RES? We also ask 3) if an intraspecific RES does exist in soy, is it coordinated with, or independent of an intraspecific LES? Lastly, we ask 4) do relationships among different suites of traits

(namely, an intraspecific LES and RES), differ across environmental gradients and/ or through plant ontogeny?

Materials and Methods

Experimental site

Our study was situated at the University of Guelph agroforestry research site located in Guelph, Ontario, Canada (43° 32' 28" N, 80° 12' 32" W, 334 m a.s.l.). Details of the site are available in multiple publications (e.g. Furze et al. 2017; Hayes et al. 2018), but in short, at the 30-ha experimental site, three different annual crop species – soybean (*G. max*), corn (*Zea mays*), and winter wheat (*Triticum aestivum*) – are planted in annual rotations alongside 10 different coniferous and deciduous tree species. Trees are planted 3-6 m apart within rows that are 800 m long, and crops are planted within the 12-15 m wide inter-row spaces (Reynolds et al. 2007; Thevathasan and Gordon 2004). In addition to agroforestry arrangements, crops are also planted as monocultures in adjacent plots situated within 300 m of the agroforestry plots. Our study was conducted from May-August 2017 when soy (*Glycine max* (Fabaceae), Variety Pioneer P90Y90) was planted in both the agroforestry and monoculture sites, at densities of ~450,000 seeds ha⁻¹ arranged in rows spaced 18 cm apart. Crops in both monoculture and agroforestry arrangements received otherwise identical management conditions including zero fertilizer inputs, rain-fed irrigation, and no-till soil management (Furze et al. 2017).

The current study is built on a previous assessment of intraspecific variation in soy leaf functional traits (Hayes et al. 2018). This design permitted sampling functional traits on individual soy plants that were located within a total of 15 different 3-m² sampling blocks (each of which located at least 25 m apart). In turn, sampling blocks were distributed evenly across the following five management conditions which were implemented in order to capture a wide breadth of intraspecific variation in soy traits: i) monoculture; ii) agroforestry, within 2 m of the tree line of hybrid poplar (*Populus tremuloides* x *P. nigra* clone DNN177); iii) agroforestry, 5-6 m away from hybrid poplar into the crop row; iv) agroforestry, within 2 m of the treeline of spruce (*Picea abies*); and v) agroforestry, 5-6 m away from spruce into the crop row (Hayes et al. 2018). This

sampling regime was then replicated at three different soy developmental stages: i) late vegetative growth (V6, sampled July 10-13, 2017); ii) pod production (R3-R4, sampled July 31-Aug. 4, 2017); and iii) late pod filling (R6, sampled Aug. 21-24, 2017) (Fehr et al. 1971). In sum, this design resulted in traits being sampled on a total of $n=134$ plants.

Functional traits and environmental variables

Our analysis focused on multiple suites of traits: i) RES traits, including average root diameter (RD, mm), specific root area (SRA, root area per unit dry mass, $\text{m}^2 \text{kg}^{-1}$), specific root length (SRL, root length per unit dry mass, m g^{-1}), and specific root tip density (SRTD, number of root tips per unit dry mass, tips g^{-1}); ii) nodule traits including total nodule dry mass (NDM, g plant^{-1}) and nodule count (NC, no. nodules plant^{-1}); iii) LES traits, including leaf mass per area (LMA, g m^{-2}), maximum photosynthetic rate on a mass basis (A_{mass} , $\text{mmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$), and leaf nitrogen (N) concentrations (% mass basis); and iv) other leaf traits associated with plant hydraulics and light capture, including leaf WUE ($\text{mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$) and leaf area (cm^2); and finally iv) stem specific density (SSD, g cm^{-3}).

Leaf trait collection protocols are outlined in detail in Hayes et al. (2018), but in short, for all leaves at all sampling times we first measured area-based maximum photosynthetic rates (A_{area}) using a Li-Cor XT 6400 portable CO_2 gas analyzer affixed with a red/blue light source (Li-Cor Biosciences, Lincoln, NE, USA). All A_{area} measurements were made between 8:00-14:00 h at saturating irradiances of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, CO_2 concentrations of 400 ppm, intercellular CO_2 partial pressures $> 200 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air, relative humidity of 50-80%, and leaf temperatures of 25-30 °C. Measurements of WUE were based on these same gas flux measurements. All gas flux leaves were then immediately collected for analyses of leaf area, leaf N, and leaf mass in the lab; values of leaf area and leaf mass were then ultimately used to derive LMA and A_{mass} values for each leaf. In sum, the only difference between the leaf trait data used by Hayes et al. (2018) and the data used in our analysis, is that we employed plant-level mean leaf trait values for our analysis here.

Following assessment of leaf traits in the field (i.e. following gas flux measurements and leaf collection), all RES and nodule traits, as well as SSD, were measured on all soy plants which were initially excavated fully by hand using a small trowel and brush to clean roots, stored at -20 °C and transported to the University of Toronto Scarborough. Plant excavation in the field occurred immediately after leaf collection. In the lab all plants were then washed by hand in deionized water to remove all debris, air-dried at 60 °C to constant mass. All nodules were then removed by hand, a subset for each plant was inspected visually for the presence of N-fixing bacteria (as per the presence of a pinkish hue within nodules that were cut open), counted and weighed to derive NC and NDM, respectively. SSD was calculated using the water displacement method on a clipped segment of the main stem ~5-cm in length extending from base of the plant.

All RES traits were then measured on entire root systems, which were rinsed by hand using deionized water and scanned at 800 dpi using a flatbed scanner. Images were then analyzed using WinRhizo Pro 2016 software (Regent Instruments, Montreal Canada) in order to derive RD, as well as total root length, total number of root tips, and total root area. Following image analyses, all root systems were dried at 60 °C to constant mass, and these data were used to derive SRA, SRL, and SRTD.

Protocol for root trait collection followed previous analyses (i.e. Isaac et al. 2017) with one notable difference. Studies focusing on root traits in woody perennials (e.g. Isaac et al. 2017; McCormack et al. 2015) generally seek to restrict analyses to “absorptive roots” only while avoiding “transporting roots”. This is generally done by following published protocols that recommend absorptive roots of perennials are situated on lateral roots, are $\leq 2\text{mm}$ diameter, and/ or are at the 3rd-or-lower order in root branching hierarchies (McCormack et al. 2015; Roumet et al. 2016). However, such protocols were not designed for annuals, and in our case of soy these protocols do not readily apply since i) all of our roots evaluated had diameters $\leq 2\text{ mm}$ and ii) none of the roots within any soy root system fell within the 4th-order or higher. Therefore, we included all roots in our analysis here. Roots of seven individual plants were damaged during excavation, therefore total sample sizes for all RES traits were $n=128$ plants.

For each soy plant we also measured a total of seven environmental variables at the time of plant harvesting. These included: i) canopy openness, ii) soil moisture, iii) soil C concentrations, iv) soil N concentrations, v) soil plant available N, vi) soil plant available P, and vii) soil pH. Data for these variables were available from previously published analyses (Hayes et al. 2018). Additional to this study, we used the same soil samples, which were air-dried and passed through 2 mm sieve, to measure soil pH in 1:10 soil to water mixture, and available P that was extracted in 1:10 soil to Bray's 1 solution, filtered through Fisher P5 filter paper and quantified colourmetrically using a flow injection analyzer (QuikChem 8500, Lachat Instruments, USA).

Statistical analysis – the extent and environmental correlates of trait variation

All data analysis was conducted using R v. 3.3.2 (R Foundations for Statistical Computing, Vienna, Austria). First, we determined if traits were best described by normal or lognormal probability distributions, using the 'fitdist' R package (Delignette-Muller and Dutang 2015). Based on these analyses, log-transformed values for all traits except SRL, SRA, leaf N, and A_{mass} were used for further analyses. Descriptive statistics for log-transformed traits included median and median absolute deviation (mad), as well as quartile-based coefficients of variation (cv) for these traits as mad/ median. Standard parametric descriptive statistics were calculated for all normally distributed traits.

We then employed a mixed-model approach to evaluate if traits varied significantly as a function of sampling time, management, and a time-by-management interaction term, and incorporating block identity as a random effect. Random effects models were fit using the 'nlme' R package (Pinheiro et al. 2016), and average trait values within each time-by-management category (along with standard errors and 95% confidence limits) were then calculated using the 'lsmeans' R package (Lenth 2016).

To understand the factors explaining variation in traits, we first used a variance decomposition procedure of Messier et al. (2010) to evaluate how the three different nested levels employed in our study design (i.e. blocks within management systems within sampling time) explain soy trait variation. To do so we parameterized a linear mixed effects model using the 'nlme' R package (Pinheiro et al. 2016), where these three

levels are included as nested random effects, and an overall intercept (or average trait value) is included as the only fixed effect. The percent variance in each trait explained by these nested levels was then derived using the ‘varcomp’ function in the ‘ape’ R package (Paradis et al. 2004).

This variance partitioning procedure was then augmented with a final mixed model analysis to understand how continuous environmental variation further explained variability in soy traits. Following the analyses by Isaac et al. (2017), we parameterized a linear mixed effects model in the ‘nlme’ R package (Pinheiro et al. 2016), where blocks within management systems within sampling time were included as nested random effects, and the following environmental variables were included as fixed effects: log-canopy openness, soil moisture, log-soil C, soil N, log-plant available N, log-plant available P, and log-pH. Lastly, we calculated two diagnostics from these models: a “fixed effects r^2 ” value (i.e. the variance in a given trait explained by environmental factors alone), and a “fixed effects + random effects r^2 value” (i.e. the variance in a given trait explained by fixed environmental factors, as well as the bested random effects) (Isaac et al. 2017). These values correspond to “marginal” and “conditional” r^2 values derived by Nakagawa and Schielzeth (2013), respectively, and were estimated through the ‘sem.model.fits’ function in the ‘piecewiseSEM’ R package (Lefcheck 2016).

Statistical analysis – multi-dimensionality in soy functional traits

We assessed relationships among all soy traits using Pearson correlation tests. Correlation tests were performed across the entire dataset (where $n=127$ plants), as well as each individual sampling period separately. Due to the large number of tests and nested nature of our study design, we assessed significant correlations as those where $p \leq 0.001$ (Forstmeier et al. 2017). We then coupled Pearson correlation tests with network graphing techniques in order to aid in the visualization of trait relationships, using the ‘qgraph’ R package (Epskamp et al. 2012).

We then used a multi-step multivariate analysis procedure to evaluate how multivariate trait syndromes varied across soy plants. First, we conducted a principal components analysis (PCA) that included all traits measured across $n=127$ soy plants.

This PCA was supplemented with a multivariate permutational analysis of variance (PerMANOVA) to assess if multivariate functional trait syndromes varied significantly as a function of sampling time, management, and a sampling-by-time interaction term (as per the results of our variance decomposition procedure; see Results below). Both the PCA and PerMANOVA analyses were implemented using the ‘vegan’ R package (Oksanen et al. 2016).

Then, following the recommendations of Dray (2008), we used the ‘testdim’ function in the ‘ade4’ R package (Dray and Dufour 2007) to assess how many PCA axes should be retained for our next analysis. Specifically, through a randomization procedure, the ‘testdim’ function estimates the significance of each of the initial axes included in our final PCA. This test indicated the presence of two significant PCA axes that explained trait covariance across the entire dataset (see Results), which upon visual inspection represented i) RES traits, SSD, WUE, and leaf area, and ii) nodule traits and the LES (see Results).

Based on this information, we conducted a multiple factor analysis (MFA) in order to evaluate the degree to which the two suites of traits were correlated with, or independent of, one another. This analysis made use of the RV coefficient, such that an RV closer to an absolute value of 1 indicates that multivariate groups of traits covary closely with each other, while an absolute RV values closer to 0 indicates little correlation among multiple suites of traits (Baraloto et al. 2010; Isaac et al. 2017). Significance of our observed RV statistic was assessed using a randomization procedure as described and implemented by Isaac et al. (2017).

Results

Intraspecific trait variation

Across our dataset, RES traits, NDM, NC, and traits associated with hydraulic function (WUE, leaf area, and SSD) were the most highly variable with cvs $\geq 26.4\%$ (with the sole exception of RD where cv was 14.3%). In comparison, variation in LES traits as well as leaf area was low with cvs $\leq 25.9\%$ (Table 1).

All traits varied significantly as a function of sampling time, management, as well as a sampling time-by-management interaction term (Fig. 1, Supporting Information Table S1). Variance decomposition analysis indicated that management explained the largest proportion of the variation (28.8-72.2%) in RES and leaf hydraulic traits as well as SSD (Table 2). Variation in NDM and NC, as well as the three LES traits, were best explained by sampling time, which accounted for 32.8-55.0% of the variance in these traits (Table 2). Block identity explained between 0.0-21.6% of the variation across all traits, but was never identified as the most important explanatory factor in these analyses (Table 2). Between 9.0-55.6% of the variation in traits was unexplained by these three nested levels, with the highest proportion of unaccounted variance remaining in all belowground traits (Table 2).

Continuous environmental variables measured within these nested levels explained 11.9-36.1% of the variation in RES traits and nodules (Table 3). Specifically, canopy openness was significantly associated with variation in all RES traits and both NDM and NC, as well as SSD (Table 3). Soil moisture was significantly positively related to only NDM and NC, while soil N and C, pH, and plant available N and P were generally not significantly correlated with RES traits (Table 3). In comparison to root traits, continuous environmental variables explained less of the variation in LES- and leaf hydraulic traits (5.7-27.8%), with canopy openness being significantly related to LMA, WUE, and leaf area, and soil moisture significantly related to leaf area and leaf N; other soil variables (pH, N, C, and plant available N and P) were unrelated to leaf traits (Table 3).

Bivariate relationships among soy traits

Across the entire dataset (where $n=127$ plants measured at all time periods), soy traits were largely correlated with one another with 45 of a possible 66 pairwise correlations being statistically significant (where $p \leq 0.001$; Fig. 2a, Supporting Information Fig. S1, Supporting Information Table S3). The exceptions to this (i.e. non-significant correlations) were systematic, insofar as that leaf N and A_{mass} were not

statistically related to most RES traits, and RD was significantly correlated only with SRL (Fig. 2a, Supporting Information Fig. S1, Supporting Information Table S3).

Traits were less strongly correlated with one another at the first sampling time (i.e. vegetative growth), with 23 of a possible 66 pairwise correlations being statistically significant (where $p \leq 0.001$; Fig. 2b, Supporting Information Table S4). This is largely owing to a complete lack of correlation between both NDM and NC with any other traits at this ontogenetic stage (Fig. 2b, Supporting Information Table S4).

At the second sampling period (i.e. initial stages of pod formation), 28 of 66 possible trait correlations were significant (where $p \leq 0.001$). At this ontogenetic stage, this trend is associated with NDM and NC beginning to show evidence of covariation with certain RES traits (namely SRTD), as well as with traits associated with plant hydraulics (i.e. SSD, WUE, and leaf area; Fig. 2c, Supporting Information Table S5).

At the final sampling period (i.e. pod filling), correlations among traits were further strengthened with 35 of a possible 66 correlations being significant (where $p \leq 0.001$; Fig. 2d, Supporting Information Table S6). At this stage, notably stronger correlations emerged among NDM and NC, and all RES traits (Fig. 2d, Table S6). At the same time two key LES traits, namely leaf N and A_{mass} , became decoupled from, and uncorrelated to, all other traits at this ontogenetic stage (Fig. 2d, Supporting Information Table S6).

Multivariate relationships among soy traits

Our initial PCA and associated test of significance identified two significant axes of variation that explained 64.7% of the variation in all soy traits evaluated here (Fig. 3a). The first PCA axis explained 45.1% of the variation in soy traits, and was positively associated with SRL, SRA, and SRTD, and negatively associated with SSD, WUE, leaf area, and RD (Fig. 3a). The second PCA axis explained 19.6% of the variation in soy traits and represented intraspecific variation in NDM and NC, as well as variation along the LES (Fig. 3a). This axis was positively associated with LMA, NDM, and NC, and negatively associated with leaf N and A_{mass} (Fig. 3a). Based on our MFA, these two axes

373 were significantly orthogonal to one another (absolute RV=0.447, $p=0.001$; Supporting
374 Information Fig. S2).

375 PerMANOVA indicated that multivariate trait syndromes in soy varied
376 significantly as a function of time, management, and a time-by-management interaction
377 term (where $p \leq 0.005$ in all cases), with these factors explaining 9.9%, 19.1%, and 12.6%
378 of the variation in traits, respectively (Supporting Information Table S7). Variation in soy
379 traits along the first PCA axis associated with RES traits, WUE, leaf area, and SSD, was
380 closely linked with management conditions (Fig. 3a), while the second PCA axis
381 representing a within-genotype LES and nodules was associated with plant ontogeny
382 (Fig. 3b).

383 Specifically, variation in traits along PCA axis 1 in response to management was
384 largely associated with monoculture plants expressing the lowest SRL, SRA, and SRTD
385 values (Fig. 1b-d), coupled with the highest RD, WUE, leaf area, and SSD values (Fig.
386 1a, 1h, 1i, and 1j, respectively). Plants in the four different agroforestry management
387 systems were largely undifferentiated from one another PCA axis 1 (Fig. 1, Fig. 2).
388 Multivariate trait variation along PCA axis 2 was associated with ontogenetic variation in
389 NDM, NC, and LES traits, particularly as plants entered the pod filling stage. During this
390 stage (i.e. sampling time three), plants showed notable increases in NDM, NC, and LMA,
391 coupled with declines in leaf N and A_{mass} (Fig. 1e-g, Fig. 2).

393 Discussion

394 Our results contribute to a small but growing body of literature indicating that
395 within crop species and genotypes, plants differentiate from one another along multiple
396 intraspecific trait spectra (Gagliardi et al. 2015; Isaac et al. 2017; Martin et al. 2017).
397 Among these groups of traits, an intraspecific LES and other aboveground traits have
398 undoubtedly garnered the most attention when evaluating the physiological and
399 biomechanical constraints on plant form and function, both in natural and domesticated
400 plants (Anderegg et al. 2018; Niinemets 2015). But here we suggest that plants within a
401 single genotype also differentiate along a belowground RES that is widely hypothesized
402 to describe variation among wild plant species (e.g. Roumet et al. 2016) or communities

(e.g. Prieto et al. 2015). While these results confirm earlier reports on woody perennials (Hajek et al. 2013; Isaac et al. 2017), our analysis contributes novel findings on how other belowground plant trophic interactions – namely root nodulation – relates to other below- and aboveground functional traits in plants (Fig. 3).

Changes in traits through plant ontogeny

Variation in leaf traits through whole-plant ontogeny, particular LES traits and those associated with hydraulic functioning, have been well documented in herbaceous and woody plants (e.g. Mason et al. 2013; Ryan et al. 2006; Sendall and Reich 2013; Thomas 2010). Research has shown a general trend of plants moving towards the “resource conserving” end of the LES through ontogeny, with plants expressing leaves with higher LMA, and lower A_{mass} and leaf N. Our analyses here, and paired detailed evaluation of LES traits in soy (Hayes et al. 2018), also demonstrate this pattern, with abrupt shifts in LES traits occurring at the pod filling stage (Fig. 1g-i). In soy, these patterns could be attributable to multiple factors including N allocation to reproductive structures (e.g. Thomas 2011), increased light availability with increasing plant size (e.g. Niinemets 2004; Williams and Phillips 1980), and/ or ontogenetic declines in both RuBP carboxylase activity and/ or chloroplast numbers (e.g. Ford and Shibles 1988; Mondal et al. 1978).

Our findings of ontogenetic increases in root nodulation (Fig. 1e-f) are also consistent with studies on seasonal NDW and NC accrual and N fixation in soy, which increase through plant development up until leaf senescence (Nasielski et al. 2015; Williams and Phillips 1980; Zhao et al. 2008). Ontogenetic increases in NDW and NC through the pod filling stage (Fig. 1e-f) have been attributable to multiple mechanisms. Arguably the most widely cited hypothesis being that ontogenetic increases in soy aboveground biomass act to reduce feedback inhibition of N fixation products (Streeter 2003; Voisin et al. 2010), while increasing the supply of C-based photosynthetic assimilates necessary for N fixation (Vance and Heichel 1991), and therefore increase NDW and NC through plant development.

Ontogenetic changes in RES traits *sensu stricto* – i.e. ontogenetic change in the traits of fine roots (*sensu* McCormack et al. 2015) that occurs independently of environmental plasticity – is less well explored in trait-based ecology particularly for annual plants (cf. Laliberte 2017; Weemstra et al. 2016). Here, all RES traits varied significantly as a function of plant developmental stage while controlling for management conditions (Supporting Information Table S2). Within any given management condition we observed ontogenetic declines in SRTD, SRL, and SRA (Fig. 1b-d, Supporting Information Table S1); average RD also visually declined through ontogeny, but within a given management treatment across ontogenetic stages did have overlapping 95% confidence intervals (Supporting Information Table S1). Ontogenetic changes in SRTD, SRA, and SRL, coupled with non-significant shifts in RD, are consistent with soy plants moving towards the “resource conserving” end of an inter- or intraspecific RES through plant development (Fort et al. 2016; Isaac et al. 2017; Ma et al. 2018; Prieto et al. 2015; Weemstra et al. 2016). One hypothesis for these ontogenetic effects could be within-plant resource re-allocation from roots to reproductive structures; experimental manipulations of nutrient sinks (e.g. reproductive structures) could further elucidate mechanistic linkages between aboveground nutrient demand and RES trait strategies.

Changes in traits across environmental gradients

In addition to ontogenetic shifts, we also observed significant increases in NDW and NC in response to increasing canopy openness and soil moisture. The extensive literature surrounding N fixation in domesticated- and wild legumes presents multiple hypotheses on the environmental controls of NDM and NC. These include: i) a negative relationship between nodules and available soil N owing to the inhibitory effects of nitrate on nodule formation (e.g. Isaac et al. 2014; Salvagiotti et al. 2008); ii) a positive relationship between temperature and N fixation owing to enhanced enzymatic activity in nodules (e.g. Houlton et al. 2008); iii) a positive relationship between soil moisture and nodulation, owing to either a) greater O₂ diffusion potential in hydrated nodules and subsequent enhancement of ATP-dependent nitrogenase activity (Fleurat-Lessard et al. 2005), or b) greater aboveground tissue growth in water sufficient conditions which

reduce feedback inhibition of N fixation (Streeter 2003); and/ or to a lesser extent iv) a positive relationship between light availability and nodule activity owing to increased aboveground plant growth which acts as an enhanced sinks for fixed N (but see Carlsson et al. 2009; Whitehead and Isaac 2012).

Formal tests of these hypotheses were beyond our study, but the significant positive relationship between both NDM and NC is consistent with water and light regulation of nodules (Table 3). In our dataset these factors explained 12-26% of the variance in NC and NDM, respectively (Table 3). While positive relationships between soil moisture and nodules are consistent in the literature (e.g. Nasielski et al. 2015; Streeter 2003), light limitation to NDM and NC is contentious (see Whitehead and Isaac 2012) so we interpret these relationships cautiously. It is possible that a positive light-NDM/ NC relationship is a proxy for temperature regulation of nodulation, which although not measured directly here, could be on the order of ~ 1.25 °C between monoculture and agroforestry (Peng et al. 2015). Moreover, average increases in NDM of ~ 0.2 g plant⁻¹ in monoculture vs. crop row agroforestry treatments, are consistent with increases in NDM observed in warming experiments (cf. Table 2 in Schweitzer and Harper 1980). Questions remain surrounding the environmental mechanism(s) controlling nodules, as well as broader methodological questions surrounding whether point sampling of soil and other environmental variables is adequate in explaining belowground trait variability. However, in our study system variation in light and associated environmental parameters is compelling in explaining the strong covariation between LES traits, NC, and NDM, across management conditions (Fig. 2a) and at later ontogenetic stages (Fig. 3d).

Consistent with previous research (Isaac et al. 2017), we found that continuous environmental variables explained very little of the variation along an intraspecific RES (Table 3). The only RES trait related to soil N was SRL, which declined significantly in response to increasing soil N (Table 3). This particular finding is consistent with plants moving towards a “resource conserving” end of an intraspecific RES with increasing soil fertility. Our results of a negative relationship between the coordinated acquisitive traits of the RES and soil N are consistent with theory from unmanaged systems, that would predict greater investment in resource acquiring root tissues when belowground resources

are limited (Prieto et al. 2015). High competition for belowground resource in agricultural systems would also limit the benefits of a high SRL, namely the ability of high SRL roots to exploit pulses of belowground resources by allowing for rapid root proliferation (Eissenstat 1991).

The dynamic nature of plant trait covariation within and across species

The vast literature evaluating patterns of above- and belowground trait covariation in plants has been crucial for understanding and predicting ecological patterns and processes at the individual plant level through to global scales. We suggest that in addition to studies exploring size-dependent variation in individual functional traits (e.g. Sendall and Reich 2013; Thomas 2010), a comprehensive understanding of trait relationships, both within- and across species, can be refined by exploring how trait covariation changes through plant ontogeny. For instance, in our analysis here LES, RES, leaf hydraulic, and nodule traits in soy appear well integrated when sampled across all ontogenetic stages (Fig. 3a), with multivariate analyses then providing evidence of coordination among LES traits and nodules which is orthogonal with respect to RES traits and hydraulic traits (Fig. 2, Fig. 3a). Such analyses inform an understanding of how soy traits co-vary along managed environmental gradients (Fig. 2a, Tables 2 and 3).

However, evaluating trait relationships within specific ontogenetic stages reveals more nuanced linkages among traits as soy plants develop. Perhaps most notable, at early stages of plant development nodules are not linked with other above- and belowground traits measured in this study, while there exist multiple significant relationships between leaf and root traits (Fig. 3b). However, at reproductive onset, a much different interpretation emerges, with nodules being highly integrated with LMA, and both hydraulic and RES traits, while other key LES traits associate with C assimilation (A_{mass} and leaf N) become decoupled from any other traits (Fig. 3d). The physiological integration of traits detected in our analyses, however, should be interpreted cautiously. Specifically, nodule trait values are measured in absolute terms and are highly dependent on whole-plant size and growth, while leaf and root traits are quantified relative to tissue mass and/ or area (i.e. are therefore independent of whole-plant size and growth patterns).

These and other disparities in units of trait expression challenge the biological interpretation of trait and trait spectra (e.g. Osnas et al. 2013). In our system, relating NDM and NDC to quantitative measures of biological N₂-fixation per unit plant mass represents a tangible next step in better integrating the physiological function of nodules into above- and belowground physiological trait spectra.

The dynamic nature of trait relationships through time, particularly within a single genotype as in our study, is consistent with traits showing differential phenotypic response to environmental variability, and/ or different changes through plant ontogeny. Exploring how single traits change in response to environmental variation has of course been the focus of extensive research. However, our results indicate that future trait-based studies at individual plant-, community, or global scales, should account for whole-plant ontogenetic variation in traits when assessing inter- or intraspecific trait spectra, and how these spectra govern rates of ecosystem functioning.

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References

Albert CH, Thuiller W, Yoccoz NG, Douzet R, Aubert S, Lavorel S (2010a) A multi-trait approach reveals the structure and the relative importance of intra- vs.

551 interspecific variability in plant traits. *Funct Ecol* 24: 1192-1201. doi:
 552 10.1111/j.1365-2435.2010.01727.x.

553 Albert CH, Thuiller W, Yoccoz NG, Soudant A, Boucher F, Saccone P, Lavorel S
 554 (2010b) Intraspecific functional variability: extent, structure and sources of
 555 variation. *J Ecol* 98: 604-613. doi: 10.1111/j.1365-2745.2010.01651.x.

556 Anderegg LD, Berner LT, Badgley G, Sethi ML, Law BE, HilleRisLambers J (2018)
 557 Within-species patterns challenge our understanding of the leaf economics
 558 spectrum. *Ecol Lett* 21.

559 Baraloto C, Paine CET, Poorter L, Beauchene J, Bonal D, Domenach AM, Herault B,
 560 Patino S, Roggy JC, Chave J (2010) Decoupled leaf and stem economics in rain
 561 forest trees. *Ecol Lett* 13: 1338-1347. doi: 10.1111/j.1461-0248.2010.01517.x.

562 Bardgett RD, Mommer L, De Vries FT (2014) Going underground: root traits as drivers
 563 of ecosystem processes. *Trends Ecol Evol* 29: 692-699. doi:
 564 10.1016/j.tree.2014.10.006.

565 Cadotte MW, Cavender-Bares J, Tilman D, Oakley TH (2009) Using phylogenetic,
 566 functional and trait diversity to understand patterns of plant community
 567 productivity. *Plos One* 4. doi: ARTN e569510.1371/journal.pone.0005695.

568 Carlsson G, Palmborg C, Jumpponen A, Scherer-Lorenzen M, Hogberg P, Huss-Danell K
 569 (2009) N₂ fixation in three perennial *Trifolium* species in experimental grasslands
 570 of varied plant species richness and composition. *Plant Ecol* 205: 87-104. doi:
 571 10.1007/s11258-009-9600-9.

572 Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O,
 573 Hobbie SE, Hoorens B, Kurokawa H, Perez-Harguindeguy N, Quested HM,
 574 Santiago LS, Wardle DA, Wright IJ, Aerts R, Allison SD, van Bodegom P,
 575 Brovkin V, Chatain A, Callaghan TV, Diaz S, Garnier E, Gurvich DE, Kazakou
 576 E, Klein JA, Read J, Reich PB, Soudzilovskaia NA, Vaieretti MV, Westoby M
 577 (2008) Plant species traits are the predominant control on litter decomposition
 578 rates within biomes worldwide. *Ecol Lett* 11: 1065-1071. doi: 10.1111/j.1461-
 579 0248.2008.01219.x.

580 Damour G, Navas ML, Garnier E (2018) A revised trait-based framework for
 581 agroecosystems including decision rules. *J Appl Ecol* 55: 12-24. doi:
 582 10.1111/1365-2664.12986.
 583 de la Riva EG, Maranon T, Perez-Ramos IM, Navarro-Fernandez CM, Olmo M, Villar R
 584 (2018) Root traits across environmental gradients in Mediterranean woody
 585 communities: are they aligned along the root economics spectrum? *Plant Soil* 424:
 586 35-48. doi: 10.1007/s11104-017-3433-4.
 587 Delignette-Muller ML, Dutang C (2015) *fitdistrplus*: an R package for fitting
 588 distributions. *J Stat Softw* 64: 1-34.
 589 Diaz S, Kattge J, Cornelissen JHC, Wright IJ, Lavorel S, Dray S, Reu B, Kleyer M, Wirth
 590 C, Prentice IC, Garnier E, Bonisch G, Westoby M, Poorter H, Reich PB, Moles
 591 AT, Dickie J, Gillison AN, Zanne AE, Chave J, Wright SJ, Sheremet'ev SN,
 592 Jactel H, Baraloto C, Cerabolini B, Pierce S, Shipley B, Kirkup D, Casanoves F,
 593 Joswig JS, Gunther A, Falczuk V, Ruger N, Mahecha MD, Gorne LD (2016) The
 594 global spectrum of plant form and function. *Nature* 529: 167-171. doi:
 595 10.1038/nature16489.
 596 Djekoun A, Planchon C (1991) Water Status Effect on Dinitrogen Fixation and
 597 Photosynthesis in Soybean. *Agron J* 83: 316-322. doi: DOI
 598 10.2134/agronj1991.00021962008300020011x.
 599 Dray S (2008) On the number of principal components: a test of dimensionality based on
 600 measurements of similarity between matrices. *Comput Stat Data An* 52: 2228-
 601 2237. doi: 10.1016/j.csda.2007.07.015.
 602 Dray S, Dufour AB (2007) The ade4 package: Implementing the duality diagram for
 603 ecologists. *J Stat Softw* 22: 1-20.
 604 Eissenstat DM (1991) On the relationship between specific root length and the rate of
 605 root proliferation - a field-study using citrus rootstocks. *New Phytol* 118: 63-68.
 606 doi: DOI 10.1111/j.1469-8137.1991.tb00565.x.
 607 Epskamp S, Cramer AOJ, Waldorp LJ, Schmittmann VD, Borsboom D (2012) qgraph:
 608 network visualizations of relationships in psychometric data. *J Stat Softw* 48: 1-
 609 18.

610 Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971) Stage of development
611 descriptions for soybeans, *Glycine max* (L) Merrill. Crop Sci 11: 929-931.

612 Fleurat-Lessard P, Michonneau P, Maeshima M, Drevon JJ, Serraj R (2005) The
613 distribution of aquaporin subtypes (PIP1, PIP2 and gamma-TIP) is tissue
614 dependent in soybean (*Glycine max*) root nodules. Ann Bot-London 96: 457-460.
615 doi: 10.1093/aob/mci195.

616 Ford DM, Shibles R (1988) Photosynthesis and other traits in relation to chloroplast
617 number during soybean leaf senescence. Plant Physiol 86: 108-111. doi: DOI
618 10.1104/pp.86.1.108.

619 Forstmeier W, Wagenmakers EJ, Parker TH (2017) Detecting and avoiding likely false-
620 positive findings - a practical guide. Biol Rev 92: 1941-1968. doi:
621 10.1111/brv.12315.

622 Fort F, Cruz P, Lecloux E, de Oliveira LB, Stroia C, Theau JP, Jouany C (2016)
623 Grassland root functional parameters vary according to a community-level
624 resource acquisition-conservation trade-off. J Veg Sci 27: 749-758. doi:
625 10.1111/jvs.12405.

626 Furze JR, Martin AR, Nasielski J, Thevathasan NV, Gordon AM, Isaac ME (2017)
627 Resistance and resilience of root fungal communities to water limitation in a
628 temperate agroecosystem. Ecology and Evolution 7: 3443-3454. doi:
629 10.1002/ece3.2900.

630 Gagliardi S, Martin AR, Virginio ED, Rapidel B, Isaac ME (2015) Intraspecific leaf
631 economic trait variation partially explains coffee performance across agroforestry
632 management regimes. Agr Ecosyst Environ 200: 151-160. doi:
633 10.1016/j.agee.2014.11.014.

634 Garcia-Palacios P, Milla R, Delgado-Baquerizo M, Martin-Robles N, Alvaro-Sanchez M,
635 Wall DH (2013) Side-effects of plant domestication: ecosystem impacts of
636 changes in litter quality. New Phytol 198: 504-513. doi: 10.1111/nph.12127.

637 Garnier E, Navas ML (2012) A trait-based approach to comparative functional plant
638 ecology: concepts, methods and applications for agroecology. A review. Agron
639 Sustain Dev 32: 365-399. doi: 10.1007/s13593-011-0036-y.

640 Hajek P, Hertel D, Leuschner C (2013) Intraspecific variation in root and leaf traits and
 641 leaf-root trait linkages in eight aspen demes (*Populus tremula* and *P.*
 642 *tremuloides*). *Frontiers in Plant Science* 4: doi: 10.3389/fpls.2013.00415.
 643 Hayes FJ, Buchanan SW, Coleman B, Gordon AM, Reich PB, Thevathasan NV, Wright
 644 IJ, Martin AR (2018) Intraspecific variation in soy across the leaf economics
 645 spectrum. *Ann Bot-London*: mcy147. doi: 10.1093/aob/mcy147.
 646 Houlton BZ, Wang YP, Vitousek PM, Field CB (2008) A unifying framework for
 647 dinitrogen fixation in the terrestrial biosphere. *Nature* 454: 327-U334. doi:
 648 10.1038/nature07028.
 649 Isaac ME, Carlsson G, Ghoulam C, Makhani M, Thevathasan NV, Gordon AM (2014)
 650 Legume performance and nitrogen acquisition strategies in a tree-based
 651 agroecosystem. *Agroecol Sust Food* 38: 686-703. doi:
 652 10.1080/21683565.2013.870630.
 653 Isaac ME, Martin AR, Virginio ED, Rapidel B, Roupsard O, Van den Meersche K (2017)
 654 Intraspecific trait variation and coordination: Root and Leaf Economics Spectra in
 655 coffee across environmental gradients. *Frontiers in Plant Science* 8. doi: ARTN
 656 119610.3389/fpls.2017.01196.
 657 Kong DL, Ma CG, Zhang Q, Li L, Chen XY, Zeng H, Guo DL (2014) Leading
 658 dimensions in absorptive root trait variation across 96 subtropical forest species.
 659 *New Phytol* 203: 863-872. doi: 10.1111/nph.12842.
 660 Kraft NJB, Godoy O, Levine JM (2015) Plant functional traits and the multidimensional
 661 nature of species coexistence. *P Natl Acad Sci USA* 112: 797-802. doi:
 662 10.1073/pnas.1413650112.
 663 Laliberte E (2017) Below-ground frontiers in trait-based plant ecology. *New Phytol* 213:
 664 1597-1603. doi: 10.1111/nph.14247.
 665 Lavorel S, Garnier E (2002) Predicting changes in community composition and
 666 ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct Ecol* 16:
 667 545-556. doi: DOI 10.1046/j.1365-2435.2002.00664.x.
 668 Lefcheck JS (2016) *piecewiseSEM*: Piecewise structural equation modeling in R for
 669 ecology, evolution, and systematics. *Methods Ecol Evol* 7: 573–579.
 670 Lenth RV (2016) Least-squares means: the R package *lsmeans*. *J Stat Softw* 69: 1-33.

671 Ma ZQ, Guo DL, Xu XL, Lu MZ, Bardgett RD, Eissenstat DM, McCormack ML, Hedin
 672 LO (2018) Evolutionary history resolves global organization of root functional
 673 traits. *Nature* 555: 94. doi: 10.1038/nature25783.

674 Martin AR, Hale CE, Cerabolini BEL, Cornelissen JHC, Craine J, Gough WA, Kattge J,
 675 Tirona CK (2018) Inter- and intraspecific variation in leaf economics traits in
 676 wheat and maize. *Aob Plants* 10: ply006.

677 Martin AR, Isaac ME (2015) Plant functional traits in agroecosystems: a blueprint for
 678 research. *J Appl Ecol* 52: 1425-1435. doi: 10.1111/1365-2664.12526.

679 Martin AR, Isaac ME (2018) Functional traits in agroecology: advancing description and
 680 prediction in agroecosystems. *J Appl Ecol* 55: 5-11. doi: 10.1111/1365-
 681 2664.13039.

682 Martin AR, Rapidel B, Roupsard O, Van den Meersche K, de M. Virginio Filho E, Mirna
 683 Barrios M, Isaac ME (2017) Intraspecific trait variation across multiple scales: the
 684 Leaf Economics Spectrum in coffee. *Funct Ecol* 31: 604–612.

685 Mason CM, Donovan LA (2015) Evolution of the leaf economics spectrum in herbs:
 686 evidence from environmental divergences in leaf physiology across *Helianthus*
 687 (Asteraceae). *Evolution* 69: 2705-2720. doi: 10.1111/evo.12768.

688 Mason CM, McGaughey SE, Donovan LA (2013) Ontogeny strongly and differentially
 689 alters leaf economic and other key traits in three diverse *Helianthus* species. *J Exp*
 690 *Bot* 64: 4089-4099. doi: 10.1093/jxb/ert249.

691 McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo DL,
 692 Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB, Leppalammi-Kujansuu J,
 693 Norby RJ, Phillips RP, Pregitzer KS, Pritchard SG, Rewald B, Zadworny M
 694 (2015) Redefining fine roots improves understanding of below-ground
 695 contributions to terrestrial biosphere processes. *New Phytol* 207: 505-518. doi:
 696 10.1111/nph.13363.

697 Messier J, McGill BJ, Lechowicz MJ (2010) How do traits vary across ecological scales?
 698 A case for trait-based ecology. *Ecol Lett* 13: 838-848. doi: 10.1111/j.1461-
 699 0248.2010.01476.x.

700 Milla R, Morente-Lopez J, Alonso-Rodrigo JM, Martin-Robles N, Chapin FS (2014)
 701 Shifts and disruptions in resource-use trait syndromes during the evolution of

herbaceous crops. Proceedings of the Royal Society B 281. doi:
10.1098/rspb.2014.1429.

Milla R, Osborne CP, Turcotte MM, Violle C (2015) Plant domestication through an
ecological lens. Trends Ecol Evol 30: 463-469. doi: 10.1016/j.tree.2015.06.006.

Mommer L, Weemstra M (2012) The role of roots in the resource economics spectrum.
New Phytol 195: 725-727. doi: 10.1111/j.1469-8137.2012.04247.x.

Mondal MH, Brun WA, Brenner ML (1978) Effects of sink removal on photosynthesis
and senescence in leaves of soybean (*Glycine max* L) plants. Plant Physiol 61:
394-397. doi: DOI 10.1104/pp.61.3.394.

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R^2 from
generalized linear mixed-effects models. Methods Ecol Evol 4: 133-142. doi:
10.1111/j.2041-210x.2012.00261.x.

Nasielski J, Furze JR, Tan J, Bargaz A, Thevathasan NV, Isaac ME (2015) Agroforestry
promotes soybean yield stability and N_2 -fixation under water stress. Agron
Sustain Dev 35: 1541-1549. doi: 10.1007/s13593-015-0330-1.

Niinemets U (2004) Adaptive adjustments to light in foliage and whole-plant
characteristics depend on relative age in the perennial herb *Leontodon hispidus*.
New Phytol 162: 683-696. doi: 10.1111/j.1469-8137.2004.01071.x.

Niinemets U (2015) Is there a species spectrum within the world-wide leaf economics
spectrum? Major variations in leaf functional traits in the Mediterranean
sclerophyll *Quercus ilex*. New Phytol 205: 79-96. doi: 10.1111/nph.13001.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL,
Solymos P, Stevens MHH, Wagner H (2016) *vegan*: Community Ecology
Package in R version 2.3-5.

Osnas JL, Lichstein JW, Reich PB, Pacala SW (2013) Global leaf trait relationships:
mass, area, and the leaf economics spectrum. Science 340: 741-744.

Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution
in R language. Bioinformatics 20: 289-290. doi: 10.1093/bioinformatics/btg412.

Peng XB, Thevathasan NV, Gordon AM, Mohammed I, Gao PX (2015) Photosynthetic
response of soybean to microclimate in 26-year-old tree-based intercropping

732 systems in Southern Ontario, Canada. Plos One 10. doi: ARTN
733 e012946710.1371/journal.pone.0129467.

734 Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RC (2016) *nlme*: Linear and Nonlinear
735 Mixed Effects Models. R package version 31-127.

736 Poorter L, Bongers F (2006) Leaf traits are good predictors of plant performance across
737 53 rain forest species. Ecology 87: 1733-1743. doi: Doi 10.1890/0012-
738 9658(2006)87[1733:Ltagpo]2.0.Co;2.

739 Prieto I, Roumet C, Cardinael R, Dupraz C, Jourdan C, Kim JH, Maeght JL, Mao Z,
740 Pierret A, Portillo N, Rounsard O, Thammahacksa C, Stokes A (2015) Root
741 functional parameters along a land-use gradient: evidence of a community-level
742 economics spectrum. J Ecol 103: 361-373. doi: 10.1111/1365-2745.12351.

743 Reynolds PE, Simpson JA, Thevathasan NV, Gordon AM (2007) Effects of tree
744 competition on corn and soybean photosynthesis, growth, and yield in a temperate
745 tree-based agroforestry intercropping system in southern Ontario, Canada. Ecol
746 Eng 29: 362-371. doi: 10.1016/j.ecoleng.2006.09.024.

747 Roucou A, Violle C, Fort F, Roumet P, Ecartot M, Vile D (2018) Shifts in plant
748 functional strategies over the course of wheat domestication. J Appl Ecol 55: 25-
749 37. doi: 10.1111/1365-2664.13029.

750 Roumet C, Birouste M, Picon-Cochard C, Ghestem M, Osman N, Vrignon-Brenas S, Cao
751 KF, Stokes A (2016) Root structure-function relationships in 74 species: evidence
752 of a root economics spectrum related to carbon economy. New Phytol 210: 815-
753 826. doi: 10.1111/nph.13828.

754 Ryan MG, Phillips N, Bond BJ (2006) The hydraulic limitation hypothesis revisited.
755 Plant Cell Environ 29: 367-381. doi: 10.1111/j.1365-3040.2005.01478.x.

756 Salvagiotti F, Cassman KG, Specht JE, Walters DT, Weiss A, Dobermann A (2008)
757 Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Field
758 Crop Res 108: 1-13. doi: 10.1016/j.fcr.2008.03.001.

759 Salvagiotti F, Specht JE, Cassman KG, Walters DT, Weiss A, Dobermann A (2009)
760 Growth and nitrogen fixation in high-yielding soybean: impact of nitrogen
761 fertilization. Agron J 101: 958-970. doi: 10.2134/agronj2008.0173x.

- Schweitzer LE, Harper JE (1980) Effect of light, dark, and temperature on root nodule activity (acetylene reduction) of soybeans. *Plant Physiol* 65: 51-56. doi: DOI 10.1104/pp.65.1.51.
- Sendall KM, Reich PB (2013) Variation in leaf and twig CO₂ flux as a function of plant size: a comparison of seedlings, saplings and trees. *Tree Physiol* 33: 713-729. doi: 10.1093/treephys/tpt048.
- Streeter JG (1985) Nitrate inhibition of legume nodule growth and activity. 2. Short-term studies with high nitrate supply. *Plant Physiol* 77: 325-328. doi: DOI 10.1104/pp.77.2.325.
- Streeter JG (2003) Effects of drought on nitrogen fixation in soybean root nodules. *Plant Cell Environ* 26: 1199-1204. doi: DOI 10.1046/j.1365-3040.2003.01041.x.
- Thevathasan NV, Gordon AM (2004) Ecology of tree intercropping systems in the North temperate region: experiences from southern Ontario, Canada. *Agroforest Syst* 61-2: 257-268. doi: DOI 10.1023/B:AGFO.0000029003.00933.6d.
- Thomas SC (2010) Photosynthetic capacity peaks at intermediate size in temperate deciduous trees. *Tree Physiol* 30: 555-573. doi: 10.1093/treephys/tpq005.
- Thomas SC (2011) Age-related changes in tree growth and functional biology: the role of reproduction. In: FC Meinzer, B Lachenbruch, TE Dawson (eds) *Size-and age-related changes in tree structure and function*. Springer, Dordrecht.
- Vance CP, Heichel GH (1991) Carbon in N₂ fixation - limitation or exquisite adaptation. *Annu Rev Plant Phys* 42: 373-392. doi: DOI 10.1146/annurev.pp.42.060191.002105.
- Violle C, Enquist BJ, McGill BJ, Jiang L, Albert CH, Hulshof C, Jung V, Messier J (2012) The return of the variance: intraspecific variability in community ecology. *Trends Ecol Evol* 27: 244-252. doi: 10.1016/j.tree.2011.11.014.
- Voisin AS, Munier-Jolain NG, Salon C (2010) The nodulation process is tightly adjusted to plant growth. An analysis using environmentally and genetically induced variation of nodule number and biomass in pea. *Plant Soil* 337: 399-412.
- Weemstra M, Mommer L, Visser EJW, van Ruijven J, Kuyper TW, Mohren GMJ, Sterck FJ (2016) Towards a multidimensional root trait framework: a tree root review. *New Phytol* 211: 1159-1169. doi: 10.1111/nph.14003.

- Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant Soil* 199: 213-227. doi: Doi 10.1023/A:1004327224729.
- Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annu Rev Ecol Syst* 33: 125-159. doi: 10.1146/annurev.ecolsys.33.010802.150452.
- Whitehead M, Isaac ME (2012) Effects of shade on nitrogen and phosphorus acquisition in cereal-legume intercropping systems. *Agriculture* 2: 12-24.
- Williams LE, Phillips DA (1980) Effect of irradiance on development of apparent nitrogen fixation and photosynthesis in soybean. *Plant Physiol* 66: 968-972. doi: DOI 10.1104/pp.66.5.968.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. *Nature* 428: 821-827. doi: 10.1038/nature02403.
- Zadworny M, McCormack ML, Zytowskiak R, Karolewski P, Mucha J, Oleksyn J (2017) Patterns of structural and defense investments in fine roots of Scots pine (*Pinus sylvestris* L.) across a strong temperature and latitudinal gradient in Europe. *Global Change Biol* 23: 1218-1231. doi: 10.1111/gcb.13514.
- Zhao YW, Xiao X, Bi DM, Hu F (2008) Effects of sulfur fertilization on soybean root and leaf traits, and soil microbial activity. *J Plant Nutr* 31: 473-483. doi: 10.1080/01904160801895001.

818 **Tables and Figure**

819 **Table 1.** Variation in leaf-, seed-, root-, stem-, and nodule traits in soybean (*Glycine max*). Maximum likelihood was used in order to
820 fit and compare both normal- and log-normal distributions for each trait individually, with the best fit highlighted in bold. Where traits
821 were better described by log-normal distributions, median and median absolute deviation (MAD) values are presented; otherwise
822 mean and standard deviations (SD) are shown.
823

Trait group	Trait	Abbrev.	<i>n</i>	Normal	Log-normal	Mean/ Median	SD/ MAD	Range	CV
Leaf Economics	Leaf nitrogen concentration	Leaf N	134	-136.4	-139.1	4.1	0.7	2.6-6.0	16.5
	Leaf mass per area	LMA	134	-496.8	-492.7	40.6	10.9	25.7-69.1	22.9
	Light-saturated photosynthetic rate (mass basis)	A_{mass}	134	83.8	79.2	0.501	0.13	0.18-0.93	25.9
Leaf hydraulics	Instantaneous water use efficiency	WUE	133	-287.0	-227.9	3.4	1.1	1.9-14.2	55.3
Leaf area	Leaf area	-	134	-478.4	-473.6	28.5	8.2	14.8-53.6	28.4
Tissue density	Stem density	-	134	100.2	134.5	0.15	0.08	0.05-0.53	60.6
Root Economics	Root diameter	RD	128	192.9	199.1	0.37	0.05	0.27-0.68	14.3
	Specific root length	SRL	128	-408.4	-410.5	13.6	5.9	3.3-32.1	43.5
	Specific root area	SRA	128	-414.4	-416.0	15.5	6.2	45.0-34.7	39.8
	Specific root tip density	SRTD	128	-1278.6	-1241.3	6869.7	3422.6	1588.3-35850.6	67.7
Nodules	Nodule count per plant	NC	134	-686.2	-660.0	54	31.1	4-230	65.0
	Nodule dry mass per plant	NDM	134	-20.2	24.2	0.22	0.2	0.01-1.5	84.9

826 **Table 2.** Variation in leaf-, seed-, root-, and stem traits in soybean (*Glycine max*) across
827 different nested levels. Log-transformed values were used, as informed by descriptive
828 statistical analysis presented in Table 1, and the nested level explaining the largest
829 proportion of variation for a given trait is highlighted in bold.

830

Trait group	Trait	<i>n</i>	Time	Management	Block	Unexplained
Leaf Economics	Leaf N	134	0.518	0.152	0.209	0.122
	log-LMA	134	0.494	0.385	0.031	0.09
	<i>A</i> _{mass}	134	0.328	0.292	0.219	0.161
Leaf hydraulics	log-WUE	133	0.115	0.636	0.16	0.089
Leaf area	log-leaf area	134	0.0	0.51	0.216	0.275
Tissue density	log-SSD	134	0.008	0.722	0.115	0.156
Root Economics	log-RD	128	0.156	0.288	0.0	0.556
	SRL	128	0.0	0.539	0.076	0.385
	SRA	128	0.0	0.559	0.103	0.338
	log-SRTD	128	0.0	0.639	0.063	0.298
Nodules	log-NC	134	0.417	0.19	0.077	0.317
	log-NDM	134	0.55	0.131	0.003	0.317

831

832

833 **Table 3.** Environmental predictors of trait variation in soy (*Glycine max*). For each trait, all parameter estimates ($\pm$ standard error)
834 were fit using linear mixed-effects models where environmental parameters (as well as an overall intercept) were included as fixed
835 effects, and block within management condition within time of sampling were included as nested random effects. Significant
836 parameters (where $p \leq 0.05$, with parameter p -values in brackets) are highlighted in bold. Also shown are the marginal and conditional
837 r^2 values that represent the variance in a given trait explained by the fixed effects alone, and the variance explained by the fixed and
838 random effects combined, respectively.
839

Trait	n	Intercept	log- Openness	Soil moisture	log-Soil C	Soil N	log-plant N	log-plant P	log- pH	Marg. r^2	Cond. r^2
Leaf N	134	0.1 $\pm$ 3.3 (0.984)	0.1 $\pm$ 0.3 (0.816)	0.1$\pm$0.02 (<0.001)	0.7 $\pm$ 0.5 (0.175)	-0.2 $\pm$ 0.3 (0.54)	0.03 $\pm$ 0.1 (0.573)	0.02 $\pm$ 0.1 (0.864)	0.4 $\pm$ 1.3 (0.769)	0.057	0.924
log-LMA	134	3.24$\pm$0.69 (<0.001)	0.4$\pm$0.03 (<0.001)	-0.01 $\pm$ 0.004 (0.104)	-0.01 $\pm$ 0.1 (0.887)	-0.02 $\pm$ 0.1 (0.665)	-0.01 $\pm$ 0.01 (0.753)	0.01 $\pm$ 0.03 (0.761)	-0.4 $\pm$ 0.3 (0.191)	0.288	0.919
A_{mass}	134	0.4 $\pm$ 0.7 (0.572)	-0.1 $\pm$ 0.1 (0.121)	0.003 $\pm$ 0.004 (0.416)	0.1 $\pm$ 0.1 (0.245)	0.03 $\pm$ 0.1 (0.558)	0.02 $\pm$ 0.01 (0.147)	-0.02 $\pm$ 0.03 (0.368)	0.02 $\pm$ 0.3 (0.952)	0.094	0.857
log-WUE	133	-2.8 $\pm$ 1.8 (0.123)	0.5$\pm$0.2 (0.025)	-0.004 $\pm$ 0.01 (0.7)	0.04 $\pm$ 0.3 (0.876)	0.1 $\pm$ 0.1 (0.709)	0.04 $\pm$ 0.03 (0.214)	0.02 $\pm$ 0.1 (0.695)	0.9 $\pm$ 0.7 (0.175)	0.208	0.928
log-leaf area	134	2.4 $\pm$ 1.7 (0.17)	0.4$\pm$0.2 (0.025)	0.03$\pm$0.01 (0.004)	0.4 $\pm$ 0.3 (0.163)	0.2 $\pm$ 0.1 (0.144)	0.01 $\pm$ 0.03 (0.821)	-0.01 $\pm$ 0.1 (0.904)	-1.0 $\pm$ 0.7 (0.141)	0.278	0.803
log-seed mass	45	2.7 $\pm$ 4.3 (0.542)	-0.4 $\pm$ 0.3 (0.238)	-0.04 $\pm$ 0.03 (0.133)	-0.2 $\pm$ 0.5 (0.605)	-0.1 $\pm$ 0.4 (0.851)	-0.01 $\pm$ 0.1 (0.875)	0.1 $\pm$ 0.1 (0.548)	-1.2 $\pm$ 1.9 (0.53)	0.317	0.821
log-seed output	45	-5.8 $\pm$ 7.8 (0.465)	1.1$\pm$0.4 (0.017)	0.06 $\pm$ 0.04 (0.158)	0.5 $\pm$ 0.8 (0.545)	0.5 $\pm$ 0.7 (0.432)	0.2 $\pm$ 0.1 (0.087)	-0.02 $\pm$ 0.3 (0.932)	1.0 $\pm$ 3.6 (0.79)	0.584	0.776

log-SSD	134	-3.2±2.4 (0.185)	1.1±0.2 (<0.001)	0.03±0.01 (0.017)	-0.2±0.3 (0.51)	-0.1±0.2 (0.436)	-0.04±0.04 (0.329)	0.2±0.1 (0.088)	-1.4±1.1 (0.178)	0.594	0.835
log-RD	127	-0.6±1.1 (0.57)	0.2±0.1 (0.001)	0.002±0.01 (0.678)	-0.2±0.1 (0.084)	0.04±0.1 (0.653)	0.003±0.02 (0.874)	0.01±0.04 (0.875)	-0.3±0.5 (0.553)	0.227	0.581
SRL	127	1.3±33.9 (0.969)	-9.3±2.8 (0.003)	0.08±0.2 (0.642)	1.6±4.7 (0.732)	-6.6±2.7 (0.016)	-0.1±0.6 (0.833)	-1.7±1.2 (0.165)	26.6±14.4 (0.069)	0.343	0.669
SRA	127	22.4±35.8 (0.533)	-10.2±2.8 (0.001)	0.1±0.2 (0.533)	-1.1±5.0 (0.835)	-4.3±2.8 (0.124)	0.1±0.7 (0.941)	-1.9±1.3 (0.139)	21.8±15.3 (0.16)	0.356	0.678
log-SRTD	127	11.2±3.2 (0.001)	-1.0±0.2 (0.0002)	-0.003±0.02 (0.835)	0.3±0.4 (0.451)	-0.3±0.3 (0.187)	-0.01±0.06 (0.837)	-0.2±0.1 (0.086)	0.9±1.4 (0.673)	0.361	0.678
log-NC	134	2.8±3.4 (0.407)	0.5±0.2 (0.009)	0.08±0.02 (<0.001)	-0.6±0.5 (0.168)	-0.5±0.3 (0.075)	0.1±0.06 (0.115)	-0.07±0.1 (0.571)	0.4±1.5 (0.804)	0.247	0.669
log-NDM	134	-2.3±4.1 (0.571)	0.7±0.2 (0.001)	0.1±0.02 (0.012)	-0.3±0.5 (0.535)	-0.5±0.3 (0.109)	0.02±0.1 (0.751)	0.07±0.2 (0.683)	-0.6±1.9 (0.766)	0.119	0.649

Fig. 1. Mean values of functional traits in soy (*Glycine max*). Presented are least square mean trait values for soy measured across three different life stages and five different management conditions. Least square means values presented here were estimated through the linear mixed model, and corresponding numerical values, standard errors, and 95% confidence intervals are presented in Supporting Information Table S2. Total sample sizes for each trait are presented in Table 1.

Fig. 2. Multivariate relationships among leaf-, root-, stem-, and nodule traits in soy (*Glycine max*). Panels represent the results of a principal components analysis (PCA) based on $n=127$ soy plants, where traits fell along two significant axes including i) an axis defined by variation in RES traits, stem specific density, leaf area, and WUE (black vectors), and ii) an axis defined by variation along the LES and nodule traits (gray vectors), as per a multiple factor analysis (where $RV=0.447$, $p<0.001$; see Supporting Information Fig. S1). Soy plants in multivariate trait space varied significantly as a function of management (represented in Panel A) and time of sampling (represented in Panel B). For clarity, dotted lines represent 95% confidence limits presented for only the monoculture management conditions (Panel A) and the pod filling stage (Panel B).

Fig. 3. Bivariate correlation networks among 10 functional traits in soy. Correlations were assessed across the entire dataset (Panel A, where $n=127$ for all tests), as well as at each sampling time individually (Panels B, C, and D, where $n=38$, 44 , and 45 , respectively). Trait nodes are coloured according to their association with the LES and nodules (gray) or the RES/ stem specific density/ water-use efficiency/ leaf area axis of variation (black), as per results of our MFA (see Fig. 2 and Supporting Information Fig. S2). Dashed gray lines connecting any two traits correspond to significant negative relationships among traits based on a Pearson correlation test (where $p\leq 0.001$), while solid black lines correspond to significant positive correlations. Associated correlation matrices are presented in Supporting Information Tables S3-S6.