Chapter 5

Linking intra- and inter-site trait variability to plant strategies: ruderals drive trait variability at high productivity only

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A slightly version of this chapter has been submitted.
ABSTRACT

Separation of trait values into intra-site (alpha component) and inter-site (beta component) variability is a powerful tool to study plant strategies and drivers of plant trait variation. Here we relate both sources of variation with the competitor-stress tolerator-ruderal (CSR) classification to understand trait patterns across and within sites. Alpha and beta values were calculated for six traits (specific leaf area, leaf nitrogen concentration, leaf phosphorus concentration, maximum height, leaf size and stem specific density) measured in The Netherlands and Ukraine (71 sites, 389 samples of 159 species). Data spanned all growth forms and common mesic to wet habitats in temperate Europe. The relation between CSR and alpha and beta trait values was quantified with weighted least squares ANOVA and Tukey post hoc tests. Variation in leaf economy traits, changes in leaf size concomitant with plant height and plant traits related to the vertical partitioning of light within communities occurred consistently across growth forms and location, while stem density relations varied with growth form, environmental limitation and disturbance regime. In agreement with the CSR theory, beta trait values of competitors and stress tolerators were separated only along gradients representing productivity. Ruderals had deviating trait values (alpha values) only at the productive end of the gradient (beta values). Ruderals’ patterns reflected the effects of disturbance in grasslands, floodplains and sites in early states of succession, while in old forests the ruderal classification did not allow to tease apart effects of disturbance and shade tolerance. By using trait gradient analysis, we have shown that understanding trait variation requires considering how plant strategies arise across growth forms, species and locations, as well as the role of disturbance and shade tolerance as drivers of trait variability within sites, particularly when productivity is high.

INTRODUCTION

Plant traits from individual plants as measured in field sampling are the final outcome of processes operating at various levels: within individuals (e.g. variation in leaf traits within a canopy (Hirose and Werger 1987)), within species (e.g. intraspecific plasticity (Albert et al. 2010)), between species (intra-site) and among sites (inter-site). Between site variability is partly due to variation in biotic and abiotic environmental controls on species traits (Ackerly 2004, Wright et al. 2005b, Ordoñez et al. 2010b). Natural communities are also commonly characterized by large between-species variability in the expression of plant traits for a given set of controls —within sites— (Wright et al. 2005b, Westoby and Wright 2006, Ordoñez et al. 2009). Although standardized sampling can be used to minimize trait variation caused by within-individual variation (Cornelissen et al. 2003), it is not possible to isolate the other sources from each other by analyzing field data alone (Ordoñez et al. 2010a).
Drivers of intra- and inter-site trait variability

To tackle this problem, the methodology developed by (Ackerly and Cornwell 2007), known as trait gradient analysis, is a very powerful tool. This method separates mean trait values of a species (instead of values for a set of individuals) in within-sites (alpha) and between-sites (beta) components. Beta trait values define the average position of a species along a trait gradient formed by mean trait values across communities, while alpha values define the average position of a species’ trait in relation to the other species with which it coexists. In this approach, alpha and beta values are characteristic of a species (not an individual) and can be robust measures to study determinants of trait variability depending purely on species characteristics.

Alpha and beta components, in analogy to the use of alpha and beta components of diversity (Lande 1996), can be used to study which plant strategies operate between and within sites and which factors determine the coexistence of species and within-site trait variability. In this method, community means are used to form a trait gradient, assuming that community means provide an integrative measure of the biotic and abiotic factors that determine community assembly. Thus, the advantage of the methodology is that it can be used when environmental data are not available or when abiotic drivers are not known or fully understood (Ackerly and Cornwell 2007). For instance, resource supply and disturbance are considered important drivers of plant trait expression, but are typically difficult to quantify accurately. Generalizations of plant responses between and within sites to resource supply and disturbance may be made by combining alpha and beta components of traits to a plant strategy scheme that i) is defined independent of the method used to estimate species trait values, ii) is based on ‘innate’ species characteristics and iii) accounts for resource supply and disturbance as drivers of plant characteristics.

The well-known and well-established CSR strategy scheme of Grime (1977) is used in this study because it incorporates these three aspects. (Grime 1977) identified three classes of plant strategies where the transition from productive to unproductive habitats is paralleled by changes in species composition from those that catch large amounts of resources and have a fast growth rate (C – competitors), towards those that are characterized by a slow and steady growth, denser tissues and long life spans (S – stress-tolerators). Disturbance restricts competitive effects and drives traits that increase investments into the reproductive phase of the life cycle, but also affects traits related to leaf phenology and photosynthesis (Grime 2006), leading to higher growth rates and shorter life cycles (R – ruderal).

Putting the CSR strategy scheme into the context of beta and alpha trait diversity, we hypothesize that the C-S axis separates species mostly along beta values, representing a gradient from poor (S) to rich (C) habitats. Ruderals (reflecting disturbance) are hypothesized to determine variability along the alpha component (variance within sites), by having more extreme trait values. A quantitative test of these expected patterns can be used to gain better insight into the processes underlying trait variability and convergence in
particular and the functional interpretation of plant strategies in general. Since the large
trait variability is one of the main obstacles hindering prediction of plant species’ responses
to abiotic factors, such insights are urgently needed. For this analysis we focus on leaf traits
that reflect important plant ecological strategic decisions: the set of functional traits that
reflect how plants catch and conserve resources: SLA (specific leaf area), LNC (leaf nitrogen
concentration), LPC (leaf phosphorus concentration) and the set of plant traits related to
the competition for light and water transport: maximum height, leaf size and SSD (stem
specific density).

MATERIALS AND METHODS

Sites selection
Plant traits were measured across 50 sites in The Netherlands (referred here as NL) and 19
sites in Ukraine (referred here as UKR), representing the dominant mesic to wet habitats in
temperate Europe. Within The Netherlands, there is little spatial variation in climate with a
mean annual precipitation of 769 mm which is evenly distributed across the year and a
mean annual temperature of 9.7°C (data from the Royal Netherlands Meteorological
Institute: [http://www.knmi.nl/klimatologie](http://www.knmi.nl/klimatologie)). The sites were chosen to span a wide range of
nutrient and water availability conditions. They included wet dune slacks, floodplains,
grasslands, forests, shrub-lands and heath, and are described in detail in Ordoñez et al.
(2010b).

The study in Ukraine was carried out in floodplains of the Transcarpathia region
(Zakarpatska province; neighbouring Hungary, Slovakia and Romania). This region harbours
some of the last surviving refuges of ancient primeval floodplain forests of Europe
(Drescher et al. 2003, Drescher and Prots 2005). As such, the forests of this study represent
some of the more pristine floodplain forests within temperate Eurasia (Klimo and Hager
2001). Climate in this region is characterised by cold snowy winters and warm humid
summers, while most floods occur in spring. The sites covered a successional gradient from
wet meadows and recently deposited river banks via well-developed Salix-dominated river
bank stands up to 200 years old stands dominated by Fraxinus and Quercus.

Plant sampling and plant traits
For sites in NL, vegetation composition and abundance was obtained from (van der Peijl et
al. 2000) or when not available, recorded at each site before sampling. For all sites in UKR,
the abundance of the species to be sampled for traits was recorded at each site, prior to
sampling. Only dominant plant species were sampled until the cover sampled amounted to
more than 50% of the total vascular plant cover. Dominant species were selected based on
the assumption that they reflect the major adaptations to the environmental conditions of
the site. A total of 71 sites (52 sites in NL and 19 in UKR), 389 species samples (282 in NL
and 107 in UKR), corresponding to 159 different species (106 in NL and 53 in UKR) and
spanning all growth forms, were collected.
Six traits were measured: SLA (m² kg⁻¹), LNC (mg g⁻¹), LPC (mg g⁻¹), leaf size (cm²), SSD, (mg mm³) and maximum plant height (m). For NL, all sampling and measurement procedures were carried out following guidelines from Cornelissen et al. (2003) and are described in detail in Ordoñez et al. (2010b). Sampling and measurements procedures in UKR were the same as those in NL, with the exception of leaf area measurements that were obtained with a LiCor leaf area meter in NL and in UKR by analysis of digital pictures of each leaf with Image J software (http://rsbweb.nih.gov/ij/index.html).

**Alpha and Beta values**

Species trait means and its alpha and beta components (alpha and beta trait values) were estimated using the methodology described in (Ackerly and Cornwell 2007). In short, for each trait, abundance weighted means for plots (eqn 1) and species (eqn 2) are defined by:

\[
p_j = \frac{\sum_{i=1}^{S} a_{ij} t_{ij}}{\sum_{i=1}^{S} a_{ij}}, \quad \text{eqn 1.}
\]

\[
t_i = \frac{\sum_{j=1}^{P} a_{ij} t_{ij}}{\sum_{j=1}^{P} a_{ij}}, \quad \text{eqn 2.}
\]

Where \(t_{ij}\) is the observed trait value and \(a_{ij}\) is the abundance of species \(i\) in plot \(j\). The total number of species in the study is \(S\) and of plots it is \(P\). The plot trait means can be considered as a measure that integrates the abiotic and biotic environment that determines community assembly. As such, plot means form a gradient of community means defined by species traits. By arranging observed trait values \((t_{ij})\) by plot means \((p_j)\), the relationship between individual observed trait values (which include intraspecific variation if traits of the same species are measured in various plots) and plot means can be studied and various parameters that characterize a species can be estimated (Ackerly and Cornwell 2007). In this plot, by definition, the ordinary least squares regression of \(t_{ij}\) vs. \(p_j\) has a slope of 1 and an intercept of 0 (X=Y line). The beta value (eqn 3) indicates the average position of a species along the trait gradient and is defined by:

\[
\beta_i = \frac{\sum_{j=1}^{P} p_j a_{ij}}{\sum_{j=1}^{P} a_{ij}} \quad \text{eqn 3.}
\]

In trait gradient analysis, alpha \((\alpha_i\) in eqn 4) and beta values \((\beta_i)\) are additive components of species trait means \((t_i\) eqn 4.) and therefore, the difference between the species means trait value and its beta value is the alpha value.

\[
t_i = \beta_i + \alpha_i \quad \text{eqn 4.}
\]

Alpha values measure the average deviation between the species trait means \(t_i\) and the plot means where the species occurs (i.e. at the plot means value that coincide with its beta value, or in other words, the distance from the X=Y line). Alpha values are therefore useful to characterize the position of a given species relative to that of co-
occurring species, in trait values. This characterisation of species’ mean trait values in terms of beta and alpha components, analogous to the analysis of diversity measures, is a strong analytical method to study determinants of trait variability among and within sites.

Prior to analysis, data of very small tree seedlings (less than 2 m) were removed from the dataset to rule out effects of ontogeny that would be difficult to evaluate (in fact, in the dataset, trait values of these small seedlings were different from the traits of adults of the same species). This led to the elimination of two Pinus sylvestris seedlings from the NL dataset so that 280 samples were included. Also prior to analysis, the distribution of the trait values was determined and traits were transformed with log_{10} (SLA, LNC, LPC, leaf size and maximum height) or a square root transformation (SSD) to attain normality. Estimation of alphas and betas was carried out using R scripts provided by (Ackerly and Cornwell 2007).

Given that alphas and betas depend on the plot means and that neither dataset included all species in a plot (as species were only sampled until the cover amounted to more than 50 %), it was necessary to first check the potential bias in alphas and betas estimated with these plot means. For this purpose we used the dataset of The Netherlands, as for this dataset the complete list of species at each site was available. For NL we created two datasets: one dataset with only the species that had been sampled and a second dataset in which the trait data of the sampled species was complemented with trait data from various trait databases (Douma, unpublished data) for species that were present in the site but not sampled for traits. Alpha and beta values were estimated for each dataset separately using both abundance weighted estimates and arithmetic means. The estimates (alpha, beta and species means) of each dataset were compared to each other for those species for that had been sampled at 3 or more sites (34 species). From these analyses it was apparent that alpha values, beta values and species means estimated by abundance weighting were not different for the sampled and the completed dataset (with only one exception for Festuca rubra), as they fell closely to the 1:1 line in a plot of species means vs. species means (B_0= 0.06, B_1= 0.95, r^2 =0.99, Festuca rubra not included), beta vs. beta values (B_0= 0.16, B_1= 0.90, r^2 =0.92) and alpha vs. alpha (B_0= -0.02, B_1= 0.87, r^2 =0.97) from each dataset (see Appendix 5-A). For estimates calculated with arithmetic means, alphas and species means had a good correspondence (species means B_0= 0.14, B_1= 0.90, r^2 =0.93; alphas B_0= -0.05, B_1= 0.93, r^2 =0.87) but betas performed less well (B_0= 0.47, B_1= 0.67, r^2 =0.70). Additionally, within datasets, weighted and arithmetic estimates of betas, alphas and species means were similar. Overall, the results of this control analysis showed that alpha and beta values estimated with abundance weighting were quite robust to sampling bias (see Appendix 5-A). Given that adding species from other data sources adds errors on which we have no control we decided to use for our analysis only the dataset with sampled species and abundance weighted estimates, correcting cover so that the sum of the cover of the sampled species equalled 100%.
Species means, beta values and alpha values were estimated for each dataset (NL and UKR) separately, in order not to confound underlying biotic and abiotic factors from these two locations, when estimating the trait gradients. The parameters from each dataset were later combined into one dataset, together with the information on the CSR-class of each species (see below).

**CSR classification**

The CSR-class (C for competitors, S for stress tolerators and R for ruderals) of every species in the dataset was obtained from a list available at [http://people.exeter.ac.uk/rh203/csr_signature.html](http://people.exeter.ac.uk/rh203/csr_signature.html) (Hunt et al. 2004). For those species that were not present in the list (14 species in NL and 14 species in UKR), the CSR-class was estimated as described in Hodgson et al. (1999) using trait information in combination with an excel sheet calculation available at: [http://people.exeter.ac.uk/rh203/allocation_csr.html](http://people.exeter.ac.uk/rh203/allocation_csr.html). For this estimation, additional trait information was obtained from online trait databases: LEDA ([http://www.leda-traitbase.org/LEDAportal/](http://www.leda-traitbase.org/LEDAportal/)) and BIOPOP ([http://www.floraweb.de/proxy/biopop/en/index.php](http://www.floraweb.de/proxy/biopop/en/index.php)), and from the trait data in the datasets itself. Still for 7 species from NL not enough information could be retrieved to estimate the CSR class. These species were included in the estimation of alphas and betas, but were excluded from the final analysis when CSR classes and alphas and betas were compared (see below).

In the CSR scheme, species can be separated into 19 classes that fit in a triangle space which includes the 100% C, S and R types at the vertices and various intermediate classes within the triangle space (for an indication of the classes see Hodgson et al. (1999)). In the datasets from NL and UKR, 17 of the 19 classes were present. Intermediate classes were attributed to C, S and/or R in proportion to their position in the triangle space using information available at: [http://people.exeter.ac.uk/rh203/csr_signature.html](http://people.exeter.ac.uk/rh203/csr_signature.html). In this manner the information of the 17 classes was reduced to the three components of the CSR.

**Data Analysis**

Pearson correlations among alpha values and among beta values of the six traits were first examined to determine how the patterns in beta and alpha values from this study corresponded with patterns reported in similar trait gradient analyses in dry environments (Ackerly and Cornwell 2007, Kooyman et al. 2010).

To test our hypothesis that the C-S axis separates species mostly along beta values (along the trait gradient) we tested the relation between CSR components and beta values. To test whether the R axis drives variability along the alpha component (variance within sites), we tested the relation of CSR components vs. alpha values and vs. absolute alpha values. We considered that, in the case of alpha values, if the average position of a CSR component was not different from 0 (i.e. from the plot mean), this might be due to deviations in alpha
values in both positive and negative directions. By testing absolute alpha values, we can make inferences as to whether a component lies more towards extreme values.

For the combined dataset of NL and UKR, relations between CSR components and alpha and beta values were tested with weighted least squares (WLS) ANOVA using SPSS 17.0. The CSR components were treated as a fixed factor and the proportional contribution of each intermediate class to the CSR component (see methods above) was the weighting factor to determine the effects of each component. Differences among marginal means of beta and alpha values for each CSR component were tested with a Tukey post-hoc test at P<0.05 as part of the WLS-ANOVA procedure.

Analyses were carried out using all species in the combined dataset (152 species, after removing the 7 species for which CSR information was not available) and using only species sampled at 3 or more sites (49 species). For both datasets, the relations of CSR components with alpha and beta values was similar in direction, but not in strength: in the smaller dataset, relations of CSR components on beta values for LNC and maximum height and on alpha values for any trait were insignificant. The similarity in the patterns from both datasets was confirmed in a control analysis in which an additional fixed factor, defining whether a species occurred at 3 or more sites, was included in the WLS-ANOVA. In this analysis, the interaction term was not significant and therefore ruled out that patterns in CSR in the group of species with n>=3 differed from those of the group of species with n<3. Given the similarity in the results from both datasets (see Appendix 5-B) and that the dataset with all species increases the power to detect patterns, we only discuss results from the dataset with all species.

Finally, we carried out a control analysis to rule out interaction effects of location (i.e. NL and UKR) with CSR components. If such effects would exist, that would imply that effects of CSR are location dependent and therefore generalization of plant strategies and the processes determining plant trait convergence and variability cannot be made. The control analysis showed that location did not have interactive effects with CSR components (Appendix 5-C). Therefore we decided not to include effects of location in further analyses.

RESULTS

Pairwise correlations of beta and alpha values in mesic to wet temperate ecosystems

Correlations of SLA vs. LNC, SLA vs. LPC and maximum height vs. leaf size for beta values from NL and UKR coincided with patterns reported previously (Ackerly and Cornwell 2007, Kooyman et al. 2010), (Figure 5.1). In general, correlations were also consistent across both datasets. The only exception was the correlation of beta values for LPC vs. LNC and LPC vs.
Drivers of intra- and inter-site trait variability | 107

![Figure 5.1. Relations between species beta trait values and species alpha trait values for pairwise combinations of SLA vs. LNC, LPC, leaf size, maximum height and SSD. Beta trait values are plotted on a logarithmic scale, while alpha values are plotted on a transformed scale (differences between species means and plot means calculated with transformed values cannot be directly back-transformed). Units and transformations are listed in the plot. Symbols represent location (NL = The Netherlands and UKR = Ukraine) and woodiness: ○ = herbaceous species in NL; ● = woody species in NL; □ = herbaceous species in UKR and □ = woody species in UKR. Unless specified otherwise, values represent Pearson correlations across growth forms and locations and their probabilities: * P < 0.05; ** P<0.01; ***P<0.001.](image-url)
Figure 5.2. Relations between species beta trait values and species alpha trait values for pairwise combinations of LPC vs. LNC, SSD vs. LPC, leaf size and maximum height and leaf size vs. maximum height. Units, symbols, and Pearson correlations follow Figure 5.1.
Drivers of intra- and inter-site trait variability

SSD for which an offset in the intercept of the relationship was apparent, reflecting differences in soil P supply between NL and UKR (Figure 5.2). The difference in LPC between the datasets did not affect other relationships.

Relationships involving beta values of SLA, maximum height, SSD and leaf size were growth form and habitat dependent. For instance, the beta of SLA was always positively correlated to leaf size and height and negatively correlated to SSD for woody species (also in Ackerly and Cornwell (2007), Kooyman et al. (2010)), but only for those herbaceous species that occurred in the understory of forests (this study). For herbaceous species commonly occurring in grasslands subjected to biomass removal by cutting or grazing, these correlations were inexistent (Figure 5.2). Across all species, beta values of SSD and maximum height were positively correlated (in contrast to Ackerly and Cornwell (2007), Kooyman et al. (2010)), and SSD and leaf size were weakly negatively correlated for woody species only.

In general, correlations among alpha values were much weaker than correlations among beta values, or were absent (Figures 1 and 2), coinciding with patterns reported before (Ackerly and Cornwell 2007). There were relatively strong correlations of alpha values involving leaf economy traits and maximum height vs. SLA (as in Ackerly and Cornwell (2007)). Relations between alpha values of SLA vs. SSD and leaf size vs. maximum height differed by growth form (i.e. only herbaceous species had significant positive correlations, woody species did not). Maximum height vs. SSD had a strong positive correlation for alpha values that was significant for both herbaceous and woody species (Figure 5.2), in contrast to Ackerly and Cornwell (2007) and Kooyman et al. (2010) in which the alpha values of these traits showed a weak negative correlation.

**Position of CSR components along trait gradients and within sites**

There was a significant separation among CSR components for all beta trait values (reflecting species positions along environmental gradients): the ruderal species occurred at the highest SLA, LNC and LPC positions along the trait gradient and at the lowest leaf size, maximum height and stem specific densities (Figure 5.3, which has been stretched horizontally to emphasize that this division occurs along the trait gradient). Stress tolerators occurred at the lower extreme position along the gradient of leaf economy traits and of SSD, but at an intermediate position in terms of leaf size and plant height. Competitors were placed at intermediate positions for leaf economy traits and SSD and occurred at the highest height and leaf size positions.

For alpha values, reflecting the relative position of a species in relation to those of co-occurring species, there were significant differences among CSR components for SLA, LNC, maximum height and SSD (Figure 5.4, which has been stretched vertically to emphasize that here the division occurs within a given ‘site’). These differences were mostly driven by ruderal species which had higher SLA (more positive values) and lower
maximum height and SSD (more negative values) than competitors and stress tolerators at a given site. Differences in LNC were driven by stress tolerators with more negative alpha values. In general, the average alpha values of CSR components did not deviated significantly from zero (only exceptions were alpha values of R for SLA and LPC) and there were no differences for alphas of LPC and leaf size (Figure 5.4). Finally, none of the analyses using absolute alpha values were significant, indicating that no group in particular occupied the extreme trait positions.

Figure 5.3. Boxplots of species beta trait values per CSR component for SLA, LNC, LPC, leaf size, maximum height and SSD. Quantiles were weighted for each CSR class by the proportion indicating the C, S and/or R position in the triangle space. CSR components (S, C and R) have been reordered to emphasize the position of the components in the beta trait gradient. Different letters next to the boxplots indicate significant differences among CSR components at the P<0.05 level as tested with a Tukey test as part of the WLS-ANOVA. F-values and probabilities for the complete model are given on top of each panel for significant models only.

In a plot of species trait values ($t_{ij}$) vs. plot means ($p_i$) (see methods), the position of a CSR component along the x-axis shows its average position along the trait gradient (i.e. the average CSR beta value), while its position on the y-axis indicates the average CSR for species mean trait values. The distance of a CSR component from to the X=Y line is the average CSR alpha value. This plot (Figure 5.5) showed that competitors and stress tolerators were separated only along the trait gradients. Competitors had higher leaf
Drivers of intra- and inter-site trait variability

economy traits, leaf size and height than stress tolerators, while the groups had a comparable SSD. Within sites, both groups were close to the plot means, as indicated by the absence of deviations from the X=Y line in the vertical direction. Ruderals were separated from competitors and stress tolerators along the gradient and within sites: ruderals were located at the high end of the gradient of SLA and had a higher SLA than its neighbours (positive alpha). The opposite pattern occurred for maximum height and SSD. For LNC and LPC, the pattern of ruderals was less pronounced than for SLA. For leaf size, ruderals were at the lower end of the gradient together with stress tolerators.

Figure 5.4. Boxplots of species alpha trait values per CSR component for SLA, LNC, LPC, leaf size, maximum height and SSD. Quantiles were weighted for each CSR class by the proportion indicating the C, S and/or R position in the triangle space. Estimation of quantiles, order of CSR components, letters above boxplots, F-values and probabilities on top of each panel follow Figure 5.3.
DISCUSSION

Robustness of alpha and beta values

The results of this study show that alpha and beta values are robust and consistent species characteristics. This is supported by results of the control analysis in which alpha and beta values were not affected by sampling bias. Moreover, the correlation patterns of alpha and beta values were consistent across the various communities, independent of location (i.e. The Netherlands and Ukraine). The conceptual definition of alpha and beta values and their consistency make them a useful tool to study the factors determining community assembly and plant strategy selection (and by consequence trait variability among and within communities).

Figure 5.5. Species mean trait values vs. plot mean trait values, averaged per CSR component, for SLA, LNC, LPC, leaf size, maximum height and SSD. Horizontal error bars represent the 95% confidence interval of the beta component of the species mean trait value (along the gradient) and vertical error bars of the alpha component (within sites). Dashed line represents the X=Y line as in a plot of observed trait values (tij) by plot means (pj), see methods. Deviations from this line are represented by the alpha component.

Consistency of plant strategies across and within communities

Intensive plant trait research in the last decade has concentrated on identifying major axes in ecological strategies (Westoby et al. 2002), which represent various trade-offs in plant functioning. Most of this research has studied trait relationships with measured trait values
Drivers of intra- and inter-site trait variability

and only recently the separation in alpha and beta values has been used to elucidate how these relationships arise from individual observations. In this study, in accordance with previous studies (Ackerly and Cornwell 2007, Kooyman et al. 2010), we show that the importance of different plant strategy axes changes with scale: correlations between beta values that imply a coupling of plant traits across sites changed in directionality or were absent when alpha values were used. This differential patterns for betas and alphas indicate that different plant strategy components are selected across compared to within sites. More importantly, in this study (with plant communities from mesic environments in temperate regions and that included almost all growth forms (ferns not included)), we show that some axes of functional variation like: i. the leaf economy spectrum (sensu Wright et al. (2004)) among and within sites; ii. the response to vertical light gradients within communities (Falster and Westoby 2005a), evidenced in the negative correlation between alpha values for SLA and maximum height and iii. the increases in leaf size with plant height among sites (Niinemets and Kull 1994, Ackerly and Cornwell 2007, Kooyman et al. 2010, Ordoñez et al. 2010b) were consistent across different growth forms and locations differing in abiotic drivers.

Other strategy components were expressed differently, depending on the interaction between environmental factors, growth form and disturbance regimes. For instance, in these temperate ecosystems, beta values of SLA only co-varied with those of maximum height and leaf size (representing communities that on average had high SLA, tall plants and large leaves) for woody species (as in Ackerly and Cornwell (2007)) and for herbaceous species that occur in the understory of forests. For these communities the increase in plot mean SLA was due to the high SLA of understory species in fertile and moist sites. Uniform disturbances such as grazing eliminated the correlation among these traits.

Also the co-variation in beta values of SSD with SLA, maximum height and leaf size was different in this study than for species that occur in dry environments: in dry communities, beta values of SSD had a strong negative relationship with beta values of SLA, leaf size and plant height reflecting plant adaptations to cope with dry environments (Ackerly 2004, Kooyman et al. 2010). In the temperate mesic ecosystems of this study, beta values of SSD were moderately and negatively related only to beta values of SLA and leaf size and only in woody species. Additionally, SSD and maximum height were positively related across all species both among communities (beta values) and within communities (alpha values). These results contrast with patterns observed in dry ecosystems where SSD and maximum height had very strong negative correlations across sites and a weak negative correlation within sites (Ackerly and Cornwell 2007, Kooyman et al. 2010). Overall, the different patterns in the functional variation of SSD, SLA, leaf size and maximum height in these wet ecosystems seem to reflect the influence of disturbance (instead of water shortage as a main driver) in selecting plant strategies. High levels of disturbance favor communities with small statures and low stem densities (grasslands, dune slacks) while as disturbance decreases, competition for light across and within sites requires taller plants.
with higher densities (as mixed forests). Thus, it seems that in these ecosystems, correlations of beta values of SSD to other traits reflected strategies more related to mechanical support than to water transport.

These results show that the trade-offs that determine the functional variation of leaf economy traits, maximum height vs. leaf size across communities and maximum height vs. SLA within sites, seem to be more constrained than those determining the functional variation of SSD in relation to other plant traits. The strength of these trade-offs became, however, apparent only by using alpha and beta values as species characteristics and thereby this result highlights the importance of trait gradient analysis when studying the determinants of plant strategies. The contrasting patterns involving correlations of SSD may be explained if we consider that SSD is related to various physiological, structural and defensive trade-offs in plant functioning (Chave et al. 2009). How these trade-offs operate under different habitats is still not well understood.

Understanding trait variability: drivers of trait convergence and divergence

The objective of combining alpha and beta values with the CSR strategy scheme was to study factors, as reflected in plant strategies, underlying trait variation across and within gradients. The CSR scheme identifies productivity (or stress at the other end of the gradient) and disturbance as the major drivers that select the species that can exist in a given habitat (Grime 1977). As such, although the CSR scheme is applied to species, this classification scheme implicitly identifies these drivers (Wilson and Lee 2000). The CSR scheme indicates that productivity is the main driver of trait values across communities, leading to trait convergence (Grime 2006). This coincides with the more general view in community ecology that habitat filtering leads to a selection of trait values as compared to the possible values from a regional pool (Keddy 1992, Cornwell et al. 2006). Disturbance, on the other hand, is considered to be the strongest driver of trait variability within sites (Grime 2006). However, also other drivers have been proposed by others. These include: alternative solutions that plants present to cope with a given level of environmental and biological limitations (Westoby and Wright 2006), spatial and temporal micro-site variability and resource partitioning (Kneitel and Chase 2004), phylogenetic or historical effects (comers vs. go-ers), biotic interactions and game theoretic or frequency dependent processes (Westoby et al. 2002) and random processes independent of trait selective advantages (Shipley 2010).

In this study, competitors and stress tolerators were separated only along the beta values. This shows the effects of productivity on trait convergence (sensu Grime (2006)) and more generally of habitat filtering (Keddy 1992, Diaz et al. 1998, Ackerly 2004, Diaz et al. 2004, Cornwell et al. 2006, Ackerly and Cornwell 2007, Kooyman et al. 2010). The lower beta trait values for stress tolerators vs. competitors (across trait gradients) reflects differences in productivity associated with soil fertility and water supply (particular for the vegetation of The Netherlands (Ordoñez et al. 2010b) and of vegetation succession
(particularly for Ukraine). Species with a more competitive character were more common in plots that on average supported taller vegetation, large leaves and intermediate SLA, LNC and LPC. Such conditions prevailed in the mixed forests of NL and UKR, although competitors also included herbaceous species common in fertile grasslands and floodplains. Thus, the patterns in beta-values for C and S were independent of growth form, consistently showing differences associated with plant strategies. At the other extreme, stress tolerating species were more common in plots that consistently had low SLA, LNC, LPC and leaf size, while their maximum height was intermediate between competitors and ruderals (e.g. heath, dune slacks and shrublands). Competitors and stress tolerators were not separated in the alpha component, although within competitors and stress tolerators herbaceous species tended to have higher alpha values than their woody counterparts.

The patterns of the alpha and beta values of ruderal species confirmed various hypothesized aspects of plant trait variability proposed in the CSR scheme (Grime 1977, Grime 2006), but also pointed to aspects of the theory which need to be reconsidered. Species with a more ruderal character were herbaceous species with high beta values for SLA, LNC, LPC and low beta values for leaf size, height and SSD, common for habitats with high fertility. In these fertile habitats, the presence of species with a ruderal component also increased trait variability because their SLA, LNC and LPC were higher and their maximum height and SSD were lower than those of their neighbors (as reflected in their alpha values). Altogether, these results confirm the prediction of the CSR scheme that productivity must be high in vegetated sites with high disturbance (Wilson and Lee 2000, Grime 2001) and provide supporting evidence for the role of disturbance in driving trait variability (Grime 2006). Still, a close inspection of the species with a ruderal component showed that these opportunistic small species with fast growth rates and short life cycles occurred in rich grasslands, floodplains (and other herbaceous communities (Pierce et al. 2007)) and in early successional stages of temperate forests. More interestingly, in mature forests on fertile sites herbaceous species with a shared ruderal and competitor component occurred (e.g. *Circaea lutetiana*, *Aegopodium podagraria*, *Alliaria petiolata*, *Glechoma hederacea*). Some of these species are opportunistic, highly plastic species that can survive and expand in a variety of environments from fully sun-exposed to deep shade, others reflect full adaptations to shade (e.g. *Circaea lutetiana*). Therefore, it seems that in forest ecosystems, the CSR components could not separate effects of disturbance (opportunistic behavior of ephemerals in the understory (Grime 2001)) from those of adaptation to shade (which should be stress tolerators according to the CSR classification (Grime 2007)). Effects of shade tolerance cannot be dismissed as they were also evident from the alpha correlations of maximum height and SLA.

These results suggest that a combination of disturbance (sensu Grime (2006)) and light partitioning (Kneitel and Chase 2004) are the drivers of within-site trait variation, but only in more fertile habitats (floodplains, grasslands and forests). This high variability
provides evidence for the general view that competition creates dispersion of traits, through limiting similarity processes (MacArthur and Levins 1967), but only at places with high productivity where competition is expected to be more intense (Weiher and Keddy 1995). Indeed, when alpha values of the six traits were plotted against soil total N (as fertility is the strongest abiotic driver in these ecosystems; (Ordoñez et al. 2010b)), a funnel shape emerged with an increasing range of trait values at increasing levels of soil total N (see Appendix 5-D). Such a funnel shape was not evident in plots of observed individual trait values vs. soil total N, because such plots include effects of factors determining variability among sites and within sites and intraspecific variation. Thus, only by isolating within-site variability through alpha values it is possible to observe its relation to abiotic factors.

**Implications for the prediction of plant traits**

Our lack of understanding -and the subsequent quantification- of processes that determine trait variability across and within plant communities is one of the major factors that hinder making adequate predictions of plant traits. By applying trait gradient analysis and combining it with the CSR scheme we are the first to quantitatively test the role of disturbance on plant trait variability within communities. Within communities, also vertical light partitioning plays an important role as a driver of plant trait variability (this study; Ackerly and Cornwell 2007). Interestingly, these two drivers seem to act only when site productivity is high. Additionally, the results of this study provide further evidence on the role of environmental filtering across gradients of productivity determined by climatic and edaphic factors (Cornwell et al. 2006, Westoby and Wright 2006, Ackerly and Cornwell 2007, Ordoñez et al. 2009, Kooyman et al. 2010). Finally, we also showed that some plant strategy components can be expressed differently, depending on the interaction between environmental factors, growth form and disturbance regimes.

These findings imply that different drivers and different plant strategy components have to be considered when predicting plant traits at different scales. For instance, for predictions at the level of species within communities, the results of this study emphasize the importance of quantifying disturbance regimes or alternatively to find proxies for the successional status of species and shade tolerance, particularly in sites with high fertility. For the time being, functional types as defined in the CSR scheme might be of use to characterize disturbance effects particularly in herbaceous communities and early stages of succession in woodlands. For established forest communities, indicators of shade tolerance such as those developed by (Niinemets and Valladares 2006) might be used. Predicting plant traits at the level of communities is more promising, given that effects of edaphic and climatic filters can be increasingly quantified (Ordonez et al., 2009). Still, this study shows that developing better predictions at these levels require improving our knowledge on the consistency of dimensions of variation across growth forms and locations with contrasting environmental conditions.
ACKNOWLEDGEMENTS

We would like to thank Will Cornwell for discussions and comments on early stages of this study on application of alpha and beta values and sampling bias. Also, warm thanks to Michaelis Michaelis Adamakis, Xiaoyuan Yu, Oksana Melchuk and Bohdan Prots for their help in collecting the data from Ukraine and James Weedon for his help with R software. This study was carried out in the framework of project A1 of the Dutch national research program Climate Change and Spatial Planning (www.klimaatvoorruiite.nl) and in the framework of the joint research program of the Dutch Water Utility sector.
Appendix 5-A. Effects of sampling bias on estimates of species means, alpha and beta

To study environmental factors modulating plant traits, plant trait responses to environmental factors and plant mediated feedbacks on ecosystem functioning, ideally all species from a plot should be sampled. However, practical limitations in time, personal and laboratory equipment available for carrying out fieldwork and laboratory analyses impose a serious limitation to the number of species sampled. Thus, we face a dilemma: sample many species for a few sites or sample many sites but fewer species per site.

Given the high variability observed in traits within a single community, average plant responses to environmental factors in natural communities can only be detected when sampling large environmental gradients, favoring the first alternative. In that case, the focus should be on dominant species because they are the ones that most likely point to the dominant factors shaping a community and that are expected to drive plant mediated feedbacks on ecosystem functioning (given their higher biomass contributions). Therefore, from a practical and theoretical point of view, it makes sense to concentrate on the dominant species and increase the number of sites sampled for studies on trait modulation by environmental factors or on plant effects on ecosystem functioning. For this reason, in this study, plant traits were measured only on dominant plant species whose cumulative cover amounted to more than 50% of the total vascular plant cover.

The method of dividing species means into its alphas and beta components might be sensitive to this sampling bias because all parameters are estimated using plot means whether or not weighted by abundance (See Ackerly and Cornwell 2007). The plot means may depend on the identity and number of species included in its estimation. For this reason, before carrying out any analysis using estimates of species means, alpha and beta values it is necessary to check whether these estimates are robust to sampling bias.

For this control analysis, we used the trait and plant abundance information from The Netherlands, given that detailed information on plant composition (abundance) was available for all plots. For the analysis we created two datasets:

- A dataset that consisted only of the sampled species, and for which the plant cover of the sampled species was corrected in such a way that the sum of the cover of the sampled species equaled 100%. (‘sampled dataset’ 280 samples, 106 species).

- A dataset in which the trait information of sampled species was complemented with trait information from various databases (Douma et al., unpublished data) for those species that were present in the site but not sampled for traits (‘completed dataset’ 697 samples, 179 species).
For specific leaf area (SLA) in each dataset, species means, alphas and betas (and also other estimates such as niche breadth and the slope representing inter-specific trait variation) were calculated by using abundance weighted means (weighted proportional to abundance) and simple arithmetic means. Species means, alphas and betas derived for those species that had been sampled at 3 or more sites (thus, n>=3 in the ‘sampled dataset’) were compared to the estimates of the same species calculated for the ‘completed dataset’, for abundance weighted estimates and arithmetic means estimates separately. If sample bias is not important, estimates from both datasets should follow a 1:1 line with linear regression estimates $b_0 = 0$ and $b_1 = 1$. We tested this by fitting an OLS (ordinary least squares) regression to a plot of estimates from the ‘sampled dataset’ vs. estimates from the ‘completed dataset’.

There were 34 species that had been sampled at 3 or more sites in the ‘sampled dataset’, from them, 33 followed closely the 1:1 line in the plot of estimates from the ‘sampled dataset’ vs. ‘completed dataset’ using abundance weighted means (Figure 5-A.1). Although the 95% confidence intervals of the $b_0$ and $b_1$ regression estimates were outside the expected values ($b_0 = 0$ and $b_1 = 1$, Table 5-A.1), the differences were small. Only one species (Festuca rubra) deviated strongly from the 1:1 line in species means and beta values, but not in alpha values. For estimates using arithmetic means, again the same species deviated strongly from the 1:1 line for species means and beta values. Although the other 33 species were not significantly different from a 1:1 line, their fit was weaker than that of abundance weighted estimates (Figure 5-A.2). This particular Festuca rubra species had been sampled at only 3 sites and all of these sites were nutrient poor sandy dune slack communities. Accordingly, the SLA of these samples was much lower than the SLA reported in online databases (Figure 5-A.3). The differences in SLA may be attributed to the effects of a deviating environment that is particularly nutrient poor. It could also be that the particular samples correspond to a subspecies (ssp. arenaria) known to be confined to the dunes.

Given that results for the other 33 species show a robust pattern, we conclude that species means, beta values and alpha values are not strongly affected by sampling bias, particularly when using abundance weighted estimates. It is important to notice that, within datasets, abundance weighted estimates are not different from non-weighted estimates. Still, for different datasets the fit for non-weighted estimates was weaker which shows that abundance weighting lowers the contribution of subdominant species to the plot means and therefore their influence on species means and its components. Altogether, these results show that sampling only dominant species (in this case up to 50% of plant cover) can be used to calculate species means, beta values and alpha values, particularly if abundance weighted estimates are used.

Finally, note that in this analysis we have concentrated only on species means, alpha and beta values. For other estimates such as niche breadth and intra-specific slopes,
sampling bias can have larger effects (as expected) and therefore for analysis involving these parameters, only complete datasets must be used.

Figure 5-A.1. Scatter plot of estimates for abundance weighted species means, beta values and alpha values from the ‘sampled dataset’ vs. estimates from the ‘completed dataset’ for specific leaf area (SLA, m² kg⁻¹). Regression lines in grey were fitted for all 34 species. Regression lines in black were fitted for 33 species, thus excluding *Festuca rubra*. For both regression lines, intercept, slopes and $r^2$ are provided.

Table 5-A.1. Summary of a simple regression of abundance weighted estimates of mean trait values, beta and alpha values of SLA from the completed dataset on the estimates of mean trait values, beta and alpha values of SLA from the sampled dataset.

<table>
<thead>
<tr>
<th></th>
<th>All species n=34</th>
<th>95% C.I.</th>
<th></th>
<th>No Festuca n=33</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression</td>
<td>Lower</td>
<td>Upper</td>
<td>$r^2$</td>
<td>Regression</td>
</tr>
<tr>
<td><strong>Species mean</strong></td>
<td>$B_0$ 0.289</td>
<td>0.132</td>
<td>0.446</td>
<td>0.83</td>
<td>$B_0$ 0.062</td>
</tr>
<tr>
<td></td>
<td>$B_1$ 0.779</td>
<td>0.652</td>
<td>0.905</td>
<td></td>
<td>$B_1$ 0.953</td>
</tr>
<tr>
<td><strong>Beta values</strong></td>
<td>$B_0$ 0.448</td>
<td>0.237</td>
<td>0.659</td>
<td>0.64</td>
<td>$B_0$ 0.151</td>
</tr>
<tr>
<td></td>
<td>$B_1$ 0.656</td>
<td>0.478</td>
<td>0.833</td>
<td></td>
<td>$B_1$ 0.898</td>
</tr>
<tr>
<td><strong>Alpha values</strong></td>
<td>$B_0$ -0.016</td>
<td>-0.025</td>
<td>-0.006</td>
<td>0.96</td>
<td>$B_0$ -0.019</td>
</tr>
<tr>
<td></td>
<td>$B_1$ 0.842</td>
<td>0.777</td>
<td>0.907</td>
<td></td>
<td>$B_1$ 0.868</td>
</tr>
</tbody>
</table>

95% Confidence intervals
Figure 5-A.2. Scatter plot of estimates for non-weighted species means, beta values and alpha values from the ‘sampled dataset’ vs. estimates from the ‘completed dataset’ for specific leaf area (SLA, m² kg⁻¹). Details are as in Figure 5-A.1.

Table 5-A.2. Summary of a simple regression of non-weighted estimates of mean trait values, beta and alpha values of SLA from the completed dataset on the estimates of mean trait values, beta and alpha values of SLA from the sampled dataset.

<table>
<thead>
<tr>
<th></th>
<th>All species n=34</th>
<th>No Festuca n=33</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% C.I.</td>
<td>95% C.I.</td>
</tr>
<tr>
<td></td>
<td>Regression</td>
<td>Lower bound</td>
</tr>
<tr>
<td>Species mean (ts)</td>
<td>B₀ 0.306</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>B₁ 0.767</td>
<td>0.644</td>
</tr>
<tr>
<td>Beta values (β)</td>
<td>B₀ 0.634</td>
<td>0.422</td>
</tr>
<tr>
<td></td>
<td>B₁ 0.535</td>
<td>0.362</td>
</tr>
<tr>
<td>Alpha values (α)</td>
<td>B₀ -0.050</td>
<td>-0.067</td>
</tr>
<tr>
<td></td>
<td>B₁ 0.890</td>
<td>0.767</td>
</tr>
</tbody>
</table>

95% Confidence intervals
Figure 5-A.3. Scatter plot of log$_{10}$ SLA of observed and estimated trait values for *Festuca rubra* vs. log$_{10}$ SLA plot means. Filled symbols represent site observations (black circles) and estimates from the online database (grey triangles); large empty symbols represent the abundance weighted species means. Sampled individuals of *Festuca rubra* in The Netherlands (lower left corner) had consistently lower SLA than the individuals from online databases (right upper corner).

Cited literature:
Appendix 5-B. Control analysis for the number of sites sampled per species

For each species, species means, beta and alpha values were calculated using the information available for that particular species from various sites. Common species tend to be dominant at more locations and were consequently sampled more often than rarer species. In case only one site was sampled for a species, its species mean value is equal to the sampled trait value while beta and alpha values depend on the mean trait value of the site in which the species occurred (thus depend on the trait values of the species with which it coexists).

We carried out an ANOVA analysis to test the relations of CSR components with alpha and beta values using all species in the combined dataset (152 species, after removing the 7 species for which CSR information was not available) and using only species sampled at 3 or more sites (49 species). For both datasets the effects of CSR class on alpha and beta values was similar in direction, but not in strength: in the smaller dataset, relations of CSR components on beta values for leaf nitrogen concentration (LNC) and maximum height and on alpha values for any trait were insignificant. We run a control analysis to test whether CSR patterns for species sampled at 3 or more sites (n>=3) were different from species sampled at fewer than 3 sites (n<3). For this analysis, a variable that defined whether a species had been sampled at 3 or more sites was created (we named this variable ‘number of observations’). The control analysis was a weighted least squares ANOVA in which CSR component was treated as fixed factor; the proportional contribution of each intermediate class to the CSR component (see ‘methods’ in the manuscript) was the weighting factor and the ‘number of observations’ was treated as a random factor. The interaction between CSR class and ‘number of observations’ was also included in the analysis to test whether effects of CSR differed according to the number of sites with observations.

The results of this analysis show that for beta values there were no interaction effects between CSR components and the number of sites with observations (Table 5-B.1). There were differences in the mean beta values of leaf phosphorus concentration (LPC) and maximum height for different CSR components and for mean beta values of LNC, LPC, leaf size and maximum height as a function of high vs. low number of sites with observations. Still, in general the ordering of the CSR components across datasets with different number of sites with observations was similar (Figure 5-B.1).

Also for alpha values, none of the interaction terms was not significant, nor were main effects of CSR component and number of site with observations significant (Table 5-B.2). There is a hint towards an interaction effect between CSR components and the number of sites with observations for alpha values of LPC and leaf size. However, given the large standard errors, these effects were not significant (Figure 5-B.2). For the other traits, alpha values of species with different number of sites with observations showed a similar
Chapter 5

A pattern for CSR means. Altogether these results showed that in general patterns in CSR effects for species with \( n \geq 3 \) were not different from those for a group of species with \( n < 3 \).

Table 5-B.1. F-values and probabilities of weighted ANOVA models to test effects of CSR component, number of sites with observations (i.e. \( n < 3 \) or \( n \geq 3 \)) and their interaction on beta values for SLA, LNC, LPC, leaf size, maximum height and stem specific density (SSD).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Predictors</th>
<th>SLA Intercept</th>
<th>Factor: CSR component</th>
<th>Factor: number observations</th>
<th>CSR component x number observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA</td>
<td></td>
<td>27,473</td>
<td>11.22</td>
<td>1.28 ( ^{ns} )</td>
<td>0.67 ( ^{ns} )</td>
</tr>
<tr>
<td>LNC</td>
<td></td>
<td>21,407</td>
<td>18.03</td>
<td>11.10 ( ^{+} )</td>
<td>0.16 ( ^{ns} )</td>
</tr>
<tr>
<td>LPC</td>
<td></td>
<td>13 ( ^{ns} )</td>
<td>21.71</td>
<td>23.96 ( ^{*} )</td>
<td>0.37 ( ^{ns} )</td>
</tr>
<tr>
<td>Leaf size</td>
<td></td>
<td>40 ( ^{*} )</td>
<td>13.29</td>
<td>16.65 ( ^{*} )</td>
<td>0.82 ( ^{ns} )</td>
</tr>
<tr>
<td>Maximum height</td>
<td></td>
<td>9 ( ^{ns} )</td>
<td>45.88</td>
<td>7.21 ( ^{*} )</td>
<td>0.22 ( ^{ns} )</td>
</tr>
<tr>
<td>SSD</td>
<td></td>
<td>5,453</td>
<td>12.55</td>
<td>3.90 ( ^{ns} )</td>
<td>0.58 ( ^{ns} )</td>
</tr>
</tbody>
</table>

Significance levels: \( ^{ns} \) not significant; \( ^{+} \) \( P < 0.1 \); \( ^{*} \) \( P < 0.05 \); \( ^{**} \) \( P < 0.01 \).

SLA: specific leaf area; LNC: leaf nitrogen concentration; LPC: leaf phosphorus concentration; SSD: stem specific density

Figure 5-B.1. Line plots for beta values by CSR component for SLA, LNC, LPC, leaf size, maximum height and SSD for species sampled at fewer than 3 sites (red circles) and for species sampled at 3 or more sites (green triangles). Error bars represent standard error of the mean.
Table 5-B.2. F-values and probabilities of weighted ANOVA models to test effects of CSR component, number of sites with observations (i.e. n<3 or n=>3) and their interaction on alpha values for SLA, LNC, LPC, leaf size, maximum height and stem specific density (SSD).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Intercepts</th>
<th>Factor: CSR component</th>
<th>Factor: number observations</th>
<th>CSR component × number observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA</td>
<td>4.502 **a</td>
<td>4.38 **</td>
<td>0.003</td>
<td>0.67 **</td>
</tr>
<tr>
<td>LNC</td>
<td>0.00 **</td>
<td>7.80 **</td>
<td>2.80 **</td>
<td>0.26 **</td>
</tr>
<tr>
<td>LPC</td>
<td>0.45 **</td>
<td>0.37 **</td>
<td>3.89 **</td>
<td>1.51 **</td>
</tr>
<tr>
<td>Leaf size</td>
<td>3.47 **</td>
<td>1.24</td>
<td>0.13</td>
<td>0.57 **</td>
</tr>
<tr>
<td>Maximum height</td>
<td>7.42 **</td>
<td>11.11+</td>
<td>8.39+</td>
<td>0.42 **</td>
</tr>
<tr>
<td>SSD</td>
<td>8.99 **</td>
<td>2.26</td>
<td>2.22</td>
<td>1.22 **</td>
</tr>
</tbody>
</table>

Significance levels: ns=not significant; + P<0.1; * P<0.05; ** P< 0.01.
SLA: specific leaf area; LNC: leaf nitrogen concentration; LPC: leaf phosphorus concentration; SSD: stem specific density

Figure 5-B.2. Line plots for alpha values by CSR component for SLA, LNC, LPC, leaf size, maximum height and SSD for species sampled at fewer than 3 sites (red circles) and for species sampled at 3 or more sites (green triangles). Error bars represent standard error of the mean.
Appendix 5-C. Control analysis for location

For this particular study we used data on various sites that represent the most dominant mesic to wet habitats in the temperate regions of Eurasia. The sites were located in The Netherlands (NL) and Ukraine (UKR). An important reason for using a strategy schemes such as the CSR is the assumption of the generality of its application. Therefore, the effects of CSR on alpha and beta values should be independent of factors such as location (i.e. UKR vs. NL).

The communities sampled in UKR corresponded mostly to pristine floodplain forests, and in NL to wet dune slacks, floodplains, grasslands, forests, shrub-lands and heath. The difference in the range of communities sampled might influence the length of the trait gradients and therefore the beta values for these two locations. For alpha values, given the large spread in trait values in all sites, differences between locations are not likely. Examination of box-plots of alpha and beta values for the 6 traits in the two locations showed that indeed the range in beta values was shorter for Ukraine, particularly for leaf phosphorus content (LPC) and leaf size (Figure 5-C.1), while the range of alpha values for both locations was similar for all six traits (Figure 5-C.2).

Differences in beta values do not represent a problem if the relation between alpha and beta values and CSR components is independent of location, so that when data from both locations are combined, the overall pattern across the trait gradient remains the same. Thus, given the shorter range of beta values in UKR it is likely that the effects of CSR are less pronounced that those in NL, but follow a similar pattern. To test whether the patterns of CSR on alpha and beta values were independent of ‘location’ (i.e. NL and UKR), we carried out a control analysis including the effects of location. For this analysis we carried out a weighted least squares ANOVA in which CSR component was treated as fixed factor, the proportional contribution of each intermediate class to the CSR component (see ‘methods’ in the manuscript) was the weighting factor and location was treated as a random factor. The interaction between CSR component and location was also included in the analysis to test whether effects of CSR differed according to location.

The results of this analysis showed that, for beta values, the interaction between CSR and location was not significant for any trait (Table 5-C.1). There were some significant differences in beta values per location for LPC, leaf size and maximum height. However, given that the variances for LPC and leaf size were heterogeneous and that the significances for the effects on LPC and leaf size were moderate (0.01<P<0.05), these results have to be treated with caution (Violation of the assumption of equality of variances in ANOVA analyses can lead to incorrectly rejecting the null hypothesis). For the interaction terms, the low F-values leave no doubt about the non-significance of these results. Despite these slight differences in means per location, the patterns in CSR for both locations were
Figure 5-C.1. Boxplots of beta values for log_{10} specific leaf area (SLA), leaf nitrogen concentration (LNC), leaf phosphorus concentration (LPC), leaf size, maximum height and square root stem specific density (SSD) for species sampled in The Netherlands (NL) and Ukraine (UKR).

Figure 5-C.2. Boxplots of alpha values for log_{10} SLA, LNC, LPC, leaf size, maximum height and square root SSD for species sampled in The Netherlands (NL) and Ukraine (UKR).
similar (Figure 5-C.3). Effects of CSR in Ukraine were less pronounced, as expected. Also for alpha values, interaction terms were not significant for any trait, nor were main effects of CSR component and location (Table 5-C.2). Alpha values of leaf size showed some tendency towards on interaction between CSR component and location. However, given the large standard errors, these effects were not significant (Figure 5-C.4). For the other traits, alpha values of species from NL and UKR showed a similar pattern in relation to CSR components. Altogether these results showed that in general patterns in relations between CSR components and alpha and beta values were independent of location. Accordingly (and to eliminate differences in variances for beta values), location was not included in further analyses.

Table 5-C.1. F-values and probabilities of weighted ANOVA models to test effects of CSR component, location (NL = The Netherlands, UKR = Ukraine) and their interaction on beta values for SLA, LNC, LPC, leaf size, maximum height and SSD.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Predictors</th>
<th>Intercept</th>
<th>Factor: CSR component</th>
<th>Factor: location</th>
<th>CSR component × location</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA</td>
<td>1,324 *</td>
<td>4.33 ns</td>
<td>25.51 *</td>
<td>0.84 ns</td>
<td></td>
</tr>
<tr>
<td>LNC</td>
<td>27,329 **</td>
<td>10.42 *</td>
<td>7.20 *</td>
<td>0.25 ns</td>
<td></td>
</tr>
<tr>
<td>LPC</td>
<td>4 ns</td>
<td>2.73 ns</td>
<td>46.80 *</td>
<td>1.71 ns</td>
<td></td>
</tr>
<tr>
<td>Leaf size</td>
<td>14 ns</td>
<td>2.30 ns</td>
<td>44.01 ns</td>
<td>1.66 ns</td>
<td></td>
</tr>
<tr>
<td>Maximum height</td>
<td>0.0 ns</td>
<td>2.94 ns</td>
<td>88.68 **</td>
<td>1.85 ns</td>
<td></td>
</tr>
<tr>
<td>SSD</td>
<td>791 *</td>
<td>2.98 ns</td>
<td>10.45 *</td>
<td>1.95 ns</td>
<td></td>
</tr>
</tbody>
</table>

Significance levels: ns=not significant; + P<0.1; * P<0.05; ** P< 0.01.
SLA: specific leaf area; LNC: leaf nitrogen concentration; LPC: leaf phosphorus concentration; SSD: stem specific density

Table 5-C.2. F-values and probabilities of weighted ANOVA models to test effects of CSR class, location (NL = The Netherlands, UKR = Ukraine) and their interaction on alpha values for SLA, LNC, LPC, leaf size, maximum height and SSD.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Predictors</th>
<th>Intercept</th>
<th>Factor: CSR component</th>
<th>Factor: location</th>
<th>CSR component × location</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA</td>
<td>7.87 ns a</td>
<td>6.03 ns</td>
<td>2.14 ns</td>
<td>1.01 ns</td>
<td></td>
</tr>
<tr>
<td>LNC</td>
<td>0.65 ns</td>
<td>4.65 ns</td>
<td>2.80 ns</td>
<td>0.44 ns</td>
<td></td>
</tr>
<tr>
<td>LPC</td>
<td>3,499 *</td>
<td>34.50 *</td>
<td>0.02 ns</td>
<td>0.04 ns</td>
<td></td>
</tr>
<tr>
<td>Leaf size</td>
<td>0.77 ns</td>
<td>1.77 ns</td>
<td>1.20 ns</td>
<td>0.38 ns</td>
<td></td>
</tr>
<tr>
<td>Maximum height</td>
<td>9.73 ns</td>
<td>9.86 *</td>
<td>8.67 *</td>
<td>1.07 ns</td>
<td></td>
</tr>
<tr>
<td>SSD</td>
<td>44.92 *</td>
<td>4.74 ns</td>
<td>0.56 ns</td>
<td>1.62 ns</td>
<td></td>
</tr>
</tbody>
</table>

Significance levels: ns=not significant; + P<0.1; * P<0.05; ** P< 0.01.
SLA: specific leaf area; LNC: leaf nitrogen concentration; LPC: leaf phosphorus concentration; SSD: stem specific density
Appendix 5-C

Figure 5-C.3. Line plots for beta values by CSR component for SLA, LNC, LPC, leaf size, maximum height and SSD for species from The Netherlands (NL, red circles) and species from Ukraine (UKR, green triangles). Error bars represent standard error of the mean.

Figure 5-C.4. Line plots for alpha values by CSR component for SLA, LNC, LPC, leaf size, maximum height and SSD for species from The Netherlands (NL, red circles) and species from Ukraine (UKR, green triangles). Error bars represent standard error of the mean.
Appendix 5-D. Alpha values of traits vs. soil total N

Figure 5-D.1. Relations between soil total N and alpha values for specific leaf Area (SLA), leaf nitrogen content (LNC), leaf phosphorus content (LPC), leaf size, maximum height and stem specific density (SSD) for sites located in the Netherlands (●) and Ukraine (△).