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When does intraspecific trait variation contribute to functional beta-diversity?

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Summary

1. Intraspecific trait variation (ITV) is hypothesized to play an important role in community assembly and the maintenance of biodiversity. However, fundamental gaps remain in our understanding of how ITV contributes to mechanisms that create spatial variation in the functional-trait composition of communities (functional β-diversity). Importantly, ITV may influence the perceived importance of environmental filtering across spatial scales.

2. We examined how ITV contributes to functional β-diversity and environmental filtering in woody plant communities in a temperate forest in the Ozark ecoregion, Missouri, USA. To test the hypothesis that ITV contributes to changes in the perceived importance of environmental filtering across scales, we compared patterns of functional β-diversity across soil-resource and topographic gradients at three spatial grains and three spatial extents. To quantify the contribution of ITV to functional β-diversity, we compared patterns that included ITV in five traits (leaf area, specific leaf area, leaf water content, leaf toughness and chlorophyll content) to patterns based on species-mean trait values.

3. Functional β-diversity that included ITV increased with spatial extent and decreased with spatial grain, suggesting stronger environmental filtering within spatially extensive landscapes that contain populations locally adapted to different habitats. In contrast, functional β-diversity based on species-mean trait values increased with spatial extent but did not change with spatial grain, suggesting weaker environmental filtering among larger communities which each contain a variety of habitats and locally adapted populations.

4. *Synthesis*. Although studies typically infer community assembly mechanisms from species-mean trait values, our study suggests that mean trait values may mask the strength of assembly mechanisms such as environmental filtering, especially in landscape-scale studies that encompass strong environmental gradients and locally adapted populations. Our study highlights the utility of integrating ITV into studies of functional β-diversity to better understand the ecological conditions under which trait variation within and among species contributes most strongly to patterns of biodiversity across spatial scales.

Key-words: community assembly, determinants of plant community diversity and structure, environmental filtering, functional diversity, Ozark oak-hickory forest, plant functional traits, spatial scale, species sorting, Tyson Research Center Plot

Introduction

Community assembly represents the outcome of multiple processes operating over multiple scales (Diamond 1975; Vellend 2010; Weiher *et al.* 2011; HilleRisLambers *et al.* 2012). To better understand the interplay of processes across scales, ecologists often examine patterns and causes of variation in community composition, known as β-diversity, a metric that links local (α) and regional (γ) patterns of biodiversity (Whittaker, Willis & Field 2001; Anderson *et al.* 2011). Recently, there has been a surge of interest in quantifying trait variation among communities (functional β-diversity) to infer the relative importance of dispersal, ecological drift and niche selection as drivers of community assembly (e.g., Swenson, Anglada-Cordero & Barone 2011; Siefert *et al.* 2013; Spasojevic, Copeland & Suding 2014). Concurrently, a growing number of theoretical and observational studies suggest that conceptual frameworks in trait-based community ecology

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should consider not only trait variation among species, but also trait variation within species across ecological gradients (Albert *et al.* 2010; Jung *et al.* 2010; Messier, McGill & Lechowicz 2010; Violle *et al.* 2012). Despite widespread interest in the causes and consequences of intraspecific trait variation (ITV) and functional β-diversity, most empirical studies have yet to integrate these concepts when testing the relative importance of multiple assembly mechanisms (Swenson, Anglada-Cordero & Barone 2011).

Intraspecific trait variation is hypothesized to play an important role in the maintenance of local diversity within ecological communities (Albert *et al.* 2011; Laughlin *et al.* 2012; Violle *et al.* 2012; Kraft *et al.* 2014). Empirical tests of this hypothesis have focused almost exclusively on local-scale processes (e.g., species interactions) that influence patterns of functional α-diversity. Because β-diversity is linked to α- and γ-diversity (e.g., $\beta = \gamma/\alpha$) (Whittaker 1960; Anderson *et al.* 2011), it is tempting to use patterns of functional α-diversity to directly infer processes that influence patterns of functional β-diversity. However, communities with similar α-diversity may have dissimilar β-diversity owing to effects of dispersal, niche selection or ecological drift on community composition. Consequently, little is known about how ITV 'scales up' to influence patterns of functional β-diversity and community assembly, especially at the landscape scale where dispersal, niche selection and ecological drift interact (Swenson, Anglada-Cordero & Barone 2011). Within a species, trait variation may result from a combination of phenotypic plasticity (Firn, Prober & Buckley 2012; Mitchell & Bakker 2014), local adaptation of traits across environmental gradients (Messier, McGill & Lechowicz 2010) or variation in local biotic interactions such as competition (Gross *et al.* 2009). This variation may contribute to patterns of functional β-diversity and inferred mechanisms of community assembly across environmental gradients. In particular, phenotypic plasticity or local adaptation can create trait variation among populations of a species arrayed along environmental gradients (Sandquist & Ehleringer 1997; Byars, Papst & Hoffmann 2007; Firn, Prober & Buckley 2012). In turn, this trait variation should increase functional β-diversity across landscapes and lead to strong relationships between functional β-diversity and local environmental conditions that vary among habitat types (Albert *et al.* 2011, 2012), thereby increasing the inferred importance of environmental filtering in community assembly. Inferences about community assembly mechanisms based on patterns of functional β-diversity are therefore likely to depend on environmental or spatial factors that underlie ITV across landscapes.

Spatial scale is a key, but often overlooked, factor that may influence when ITV contributes to functional β-diversity and environmental filtering (Albert *et al.* 2011; Barton *et al.* 2013). While the contribution of ITV to overall patterns of trait variation is hypothesized to decrease from regional to global scales (Albert *et al.* 2011), little is known about how ITV contributes to patterns of functional β-diversity at landscape scales typical of most studies of community assembly. In these studies, β-diversity may be strongly influenced by two distinct components of scale: spatial extent and spatial grain (Fig. 1, Wiens 1989; Barton *et al.* 2013). Spatial extent describes variation in the overall sampling extent of a given study while maintaining a fixed area for sampling units, whereas spatial grain describes variation in the area of a sampling unit within a fixed spatial extent (Wiens 1989; Nekola & White 1999). Importantly, these two components of scale are likely to have contrasting influences on how ITV contributes to patterns of functional β-diversity and inferred assembly mechanisms. Larger spatial extents typically contain a broader range of environmental conditions (Soininen, McDonald & Hillebrand 2007; Morlon *et al.* 2008) and likely include populations locally adapted to different habitats across environmental gradients (e.g., Messier, McGill & Lechowicz 2010; Fig. 1). Thus, we expect ITV to contribute strongly to functional β-diversity at larger spatial extents. In contrast, the contribution of ITV to functional β-diversity may decline with increasing spatial grain because larger communities are more likely to contain a variety of habitats with locally adapted populations (Fig. 1). In this case, the contribution of ITV will likely depend on the degree to which a given grain size captures habitat associations of species across landscapes (Legendre *et al.* 2009; De Caceres *et al.* 2012; Fig. 1). In the extreme case where species sorting among habitats results in taxonomically unique assemblages at each sampling grain, ITV would contribute little to functional β-diversity and the inferred importance of environmental filtering.

In this study, we examined how ITV contributes to functional β-diversity and inferred assembly mechanisms across spatial scales. We quantified ITV in five leaf traits (leaf size, specific leaf area (SLA), leaf water content (LWC), leaf toughness and chlorophyll content) measured across 681 individual saplings of 35 woody species in a temperate forest-dynamics plot in the Missouri Ozarks, USA. To test the hypothesis that ITV contributes to changes in the perceived importance of environmental filtering across scales, we compared patterns of functional β-diversity across soil-resource and topographic gradients at three spatial grains and three spatial extents. To quantify the contribution of ITV to functional β-diversity, we compared patterns that included ITV among local populations distributed across habitats with dissimilar environmental conditions to patterns based on species-mean trait values. Finally, we used variation-partitioning analyses to compare the extent to which both patterns might reflect a shift in the relative importance of assembly mechanisms across environmental and spatial gradients.

Materials and methods

STUDY SITE: THE TYSON RESEARCH CENTER PLOT

Our study was conducted at the Tyson Research Center Plot (TRCP), a 25-ha (500 × 500 m) forest-dynamics plot located at Washington University's Tyson Research Center near St. Louis, Missouri (38°31′ N, 90°33′ W; mean annual temperature 13.5 °C; mean annual precipitation 957 mm). The 800-ha research centre is located on the north-eastern edge of the Ozark ecoregion and includes strong

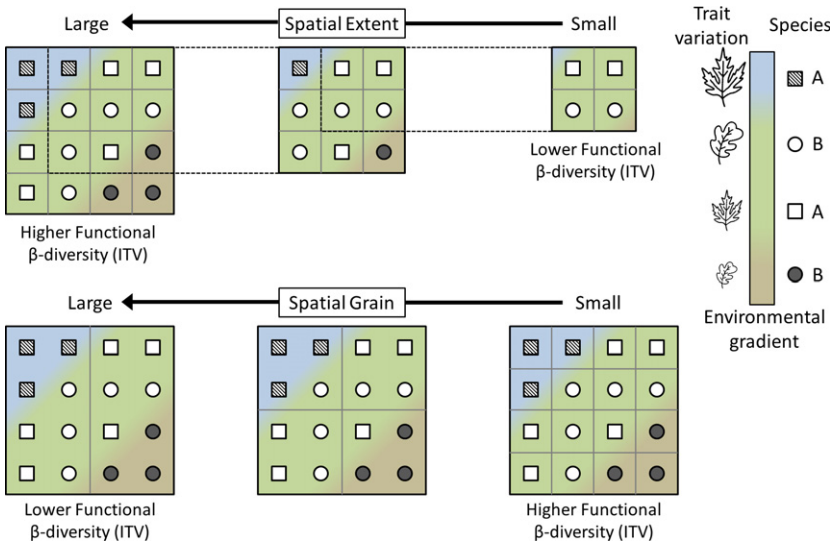

Fig. 1. An illustration of how intraspecific trait variation (ITV) may contribute to patterns of functional β-diversity and inferred community assembly mechanisms across spatial scales. From right to left, the top panels show increasing spatial extent, whereas the bottom panels show increasing spatial grain, in a simplified landscape that contains two species [species A (squares) and species B (circles)] distributed across an environmental gradient (blue, green and brown habitats). Within each panel, functional β-diversity represents the variation in traits among local communities (grey squares). Within each species, a functional trait (leaf size; shaded vs. white circles and squares) varies across the environment gradient, reflecting local adaptation of populations to different habitat types. As spatial extent increases, environmental filtering of local populations increases, resulting in a stronger contribution of ITV to functional β-diversity. As spatial grain increases, in contrast, each community contains a variety of habitats and more locally adapted populations, resulting in a weaker contribution of ITV to functional β-diversity.

edaphic and topographic gradients characteristic of oak-hickory forests in this ecoregion. The TRCP is part of a global network of forest-ecology plots monitored through the Smithsonian Center for Tropical Forest Science and Global Forest Earth Observatory (CTFS-ForestGEO; Anderson-Teixeira *et al.* 2015). For this study, we use data from a 12-ha (460 × 260 m) section of the TRCP that was censused from 2011 to 2012, in which all free-standing stems of woody species >1 cm diameter at breast height (d.b.h.) have been identified, tagged, measured and mapped following CTFS-ForestGEO protocols (Condit 1998). Additional information about the study site is available in Spasojevic *et al.* (2014).

INTRASPECIFIC TRAIT VARIATION ACROSS ENVIRONMENTAL GRADIENTS

To quantify the contribution of ITV to patterns of functional β-diversity, we measured five leaf traits on 35 tree and shrub species across four major habitat types in the TRCP: east-facing slopes, ridges, west-facing slopes and valleys (Fig. 2). First, we assigned a habitat type to each of the 299 20 × 20 m quadrats (comprising the largest spatial extent and smallest spatial grain) based on aspect and elevation using a Ward cluster analysis in JMP 10.1 (SAS Institute Inc., Cary, NC, USA), resulting in 133 quadrats on east-facing slopes, 40 quadrats on ridges, 133 quadrats on west-facing slopes and 38 quadrats in valleys (Fig. 2). This approach allowed us to quantify variation in mean trait values among habitats, but does not capture the entire range of ITV among all individuals in the plot. Owing to the logistical difficulties of measuring traits on all individuals (>18 000 individuals in the 12-ha section of the plot), we were unable to use an individual-based approach to quantify the contribution of ITV to patterns of functional beta-diversity. Our habitat-based approach therefore provides a conservative estimate of the importance of ITV in this forest. Secondly, we measured leaf size, SLA, LWC, leaf toughness and chlorophyll content on 681 individuals distributed across the four habitat types. Leaf size is associated with leaf energy and water balance and stress tolerance (Perez-Harguindeguy *et al.* 2013). Specific leaf area is associated with resource uptake strategy and tissue nitrogen (N) (Reich, Walters & Ellsworth 1997). Leaf water content is associated with relative growth rate (Garnier & Laurent 1994) and tolerance to low water availability (Farooq *et al.* 2009). Chlorophyll content is highly correlated with tissue N (Markwell & Blevins 1999). Leaf toughness is associated with protection from physical damage (Perez-Harguindeguy *et al.* 2013). In a previous study, we found that variation in these traits (e.g., SLA, leaf size) correlated with topographic and soil-resource gradients at this site (Spasojevic *et al.* 2014).

Quantifying the contribution of ITV to patterns of β-diversity is complicated by the fact that ITV can emerge through a combination of ITV across environmental gradients (Jung *et al.* 2010; Violle *et al.* 2012) and ITV across ontogenetic stages (Poorter 2007; Spasojevic *et al.* 2014; Yang *et al.* 2014). To minimize the influence of ontogenetic variation on ITV and to focus on sources of ITV that arise across environmental gradients, we restricted our sampling to understorey individuals (saplings and small-stature trees and shrubs) ≤10 cm d.b.h. For each individual, we collected three fully developed leaves with minimal damage or senescence from the upper crowns and fully hydrated the leaves in water tubes. In the laboratory, we weighed each leaf to obtain fresh mass and scanned them using a digital scanner. We measured chlorophyll content as the average of measurements taken at three locations on each leaf, avoiding the mid-rid, using an Opti-Sciences CCM-200 PLUS chlorophyll meter (Hudson, NH, USA). We measured leaf toughness (strength to punch) as the force (kg) required for a penetrometer to punch through a leaf. We measured leaf area (cm^2) from scanned leaves and petioles using ImageJ (Rasband 2007). For species with compound leaves, we calculated leaf area as the mean leaflet area per leaf including petioles (Perez-Harguindeguy *et al.* 2013). We calculated SLA ($cm^2\ g^{-1}$) as leaf area per unit dry mass after leaves were dried in an oven at 60 °C for 4 days. Lastly, we calculated LWC as the ratio of fresh mass to dry mass (Garnier *et al.* 2001).

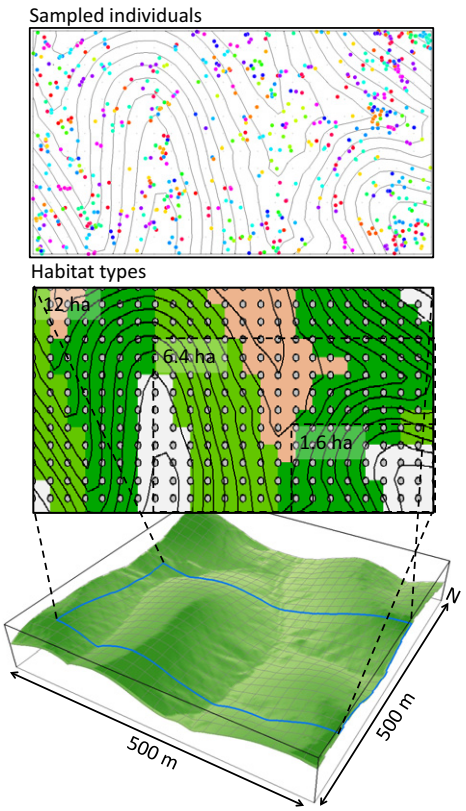

Fig. 2. Habitat variation in the Tyson Research Center Plot, Missouri, USA. The bottom map shows topographic variation from a digital elevation model based on lidar data, with 20 × 20 m quadrats overlaid as a grid. Elevation ranges from 172 to 233 m (mean = 206 m) and slope ranges from 0.8 to 26.9° (mean = 13.8°) at this spatial grain. The blue outline shows the 12-ha section (460 × 260 m) used in this study. The centre map shows four habitat types: ridges (white), valleys (beige), east-facing slopes (light green) and west-facing slopes (dark green) defined by cluster analysis which are included in all three spatial extents (1.6, 6.4 and 12 ha) used in the analysis. The top map shows locations of 681 individual trees sampled for measurement of intraspecific trait variation across habitats. Each colour represents a different species (n = 35 total species). Bottom map credit: Francis J. Baum, GIS Certificate Program, Washington University in St. Louis.

For each species, we sampled individuals in each habitat type in which it occurred. Sample sizes varied among species based on their abundance in each habitat type (n = 1–57 total individuals per species; n = 1–28 total individuals per species per habitat type). Among species, the total number of individuals sampled was strongly correlated with the total number of individuals <10 cm d.b.h. in the plot ($P < 0.001$, $r^2 = 0.77$). Ten species had less than five total individual stems across the entire 12-ha plot and all of those individuals were sampled for traits. Although these individuals likely have a small influence on functional β-diversity, it is useful to include them in community-level analyses that weight mean trait values by species abundances (described below).

STATISTICAL ANALYSES

To describe the amount of ITV within each habitat type for each trait, we decomposed the total community trait variation within each habitat into 'between-species variance' and 'within-species variance' following the method outlined in Leps *et al.* (2006). To quantify the contribution of ITV to functional β-diversity, we compared patterns of functional β-diversity that includes ITV among habitat types (ITV β-diversity) to patterns of β-diversity based on species-mean trait values (species-mean β-diversity). We examined patterns of ITV β-diversity and species-mean β-diversity at three spatial extents (1.6, 6.4 and 12 ha) and three spatial grains (20 × 20 m, 40 × 40 m and 60 × 60 m) at the largest spatial extent. The three spatial extents included a total of 40, 160 and 299 20 × 20 m quadrats, respectively. The three spatial grains included a total of 28 60 × 60 m quadrats, 66 40 × 40 m quadrats and 299 20 × 20 m quadrats, respectively. We also examined patterns of taxonomic β-diversity at each spatial extent and spatial grain to aid in the interpretation of our results.

We calculated functional β-diversity as the dissimilarity among quadrats in community weighted mean (CWM) trait values (Swenson *et al.* 2012; Spasojevic, Copeland & Suding 2014). First, we standardized values of each trait using Z-scores (mean = 0, standard deviation = 1) to control for differences in variance and units of measurement among traits and log-transformed the Z-scores to meet the assumptions of normality (when needed). Secondly, for each quadrat at each spatial extent and spatial grain, we calculated CWM trait values as the sum across all species of species' trait values weighted by their relative abundance (Garnier *et al.* 2004). For calculations of ITV β-diversity, we calculated CWM trait values of each quadrat using habitat-specific trait values. If a quadrat comprised a single habitat type (e.g., at the 20 × 20 m grain size), we calculated CWM trait values using species' trait values averaged across all individuals sampled in that habitat type. If a quadrat comprised multiple habitat types (e.g., at the 60 × 60 grain size), we calculated CWM trait values using species' trait values averaged across all individuals sampled in all of the habitat types represented in the quadrat. To quantify species-mean β-diversity, we calculated CWM trait values of each quadrat using species' trait values averaged across all individuals sampled in all habitats in which a species occurred.

We calculated β-diversity as the average distance-to-centroid, measured as the average distance (i.e., functional or taxonomic dissimilarity) from an individual quadrat to the centroid of the group of all quadrats at a given spatial extent or grain, using the 'betadisper' function in the R vegan package (Oksanen *et al.* 2010). For taxonomic β-diversity, we measured distance-to-centroids using Bray–Curtis distances. For ITV β-diversity and species-mean β-diversity, we measured distance-to-centroids using Euclidean distances based on CWM trait values of all five traits (standardized prior to analysis). For simplicity, we focus here on overall patterns of multivariate functional composition calculated using all five traits combined, rather than separate patterns for each individual trait. Although this approach may obscure the functional response of individual traits (Spasojevic & Suding 2012), it provides insights into how the overall functional composition of communities responds to environmental and spatial gradients. We tested for differences in ITV β-diversity, species-mean β-diversity and taxonomic β-diversity among spatial extents and spatial grains using a nonparametric analysis of variance based on distance-to-centroid values (Anderson *et al.* 2011).

To test the hypothesis that ITV contributes to changes in the perceived importance of environmental filtering across scales, we compared the extent to which each metric of β-diversity (ITV, species-mean, taxonomic) was explained by environmental and spatial variables using distance-based redundancy analysis (dbRDA; Peres-Neto *et al.* 2006; Legendre *et al.* 2009). Following the methods described in Spasojevic *et al.* (2014), we calculated 18 environmental

variables for each quadrat including 14 soil variables and four topographic variables. Soil variables included available N (the sum of KCl-extractable ammonium and nitrate), N mineralization rate, base saturation, effective cation exchange capacity (ECEC), exchangeable cations (Al, Ca, Fe, K, Mg, Mn, Na) determined by extraction in 0.1 M $BaCl_2$, pH (1:2 soil to solution ratio), plant-available phosphorus (P) determined by extraction in Bray-1 solution and total exchangeable bases (the sum of Ca, K, Mg and Na). Topographic variables included aspect, convexity, mean elevation and slope. Spatial variables included spatial eigenfunctions (see Appendix S2 in Supporting Information) obtained from principal components of neighbour matrices which describe spatial structure among quadrats (Borcard & Legendre 2002). Following Blanchet, Legendre & Borcard (2008), we used dbRDA to partition variation in β-diversity into individual fractions explained by pure (spatially unstructured) environmental variables, spatially structured environmental variables and spatial variables. We then performed forward-model selection using the 'Forward.sel' function in the R packfor package (Dray, Legendre & Blanchet 2007). Environmental and spatial variables retained after forward-model selection (see Appendix S2) were used to partition variation in β-diversity into the individual fractions listed above. We performed these analyses for each metric of β-diversity at each spatial extent and spatial grain.

Results

PATTERNS OF INTRASPECIFIC TRAIT VARIATION

The relative influence of ITV on the total trait variation within a habitat varied among traits and habitats (Fig. S1). In general, ITV in leaf area and LWC contributed the least to the total trait variation across all four habitat types (up to 30% and 35%, respectively) and ITV in chlorophyll content and leaf toughness contributed the most (up to 51% and 46%, respectively). On average, ITV contributed the most to the total trait variation within a habitat on ridges (40%) and the least on east-facing slopes (35%).

PATTERNS OF β-DIVERSITY ACROSS SPATIAL SCALES

β-Diversity changed systematically across spatial scales. However, the direction and strength of these changes differed for patterns of β-diversity that included ITV compared to patterns of β-diversity based on species-mean trait values. Mean ITV β-diversity increased by 88% from the smallest spatial extent to the largest spatial extent (i.e., from small to large sampling areas with a fixed spatial grain; $F_{2,496} = 16.81$, $P = 0.001$, Fig. 3a) and decreased by ~25% from smallest spatial grain to the largest spatial grain (i.e., from small to large sampling units within a fixed spatial extent; $F_{2,390} = 6.16$, $P = 0.005$, Fig. 4a). In contrast, species-mean β-diversity increased to a lesser degree (66%) from the smallest spatial extent to the largest spatial extent ($F_{2,496} = 11.59$, $P = 0.001$, Fig. 3b) and did not differ significantly across spatial grains ($F_{2,390} = 0.06$, $P = 0.95$, Fig. 4b). Overall patterns of taxonomic β-diversity paralleled patterns of ITV β-diversity, but showed smaller changes between small and large spatial extents ($F_{2,496} = 5.42$, $P = 0.006$, Fig. 5a) and small and large spatial grains ($F_{2,390} = 17.37$, $P = 0.001$, Fig. 5b).

ENVIRONMENTAL AND SPATIAL INFLUENCES ON β-DIVERSITY ACROSS SPATIAL SCALES

Environmental and spatial variables had contrasting influences on ITV β-diversity at different spatial scales. The total amount of variation in ITV β-diversity explained by environmental and spatial variables increased from the smallest spatial extent to the largest spatial extent (57% vs. 76% total explained variation, respectively; Fig. 3c). Similarly, the total amount of explained variation increased from the smallest spatial grain to the largest spatial grain (Fig. 4c), but the overall increase was smaller (76% vs. 85%, respectively) than the increase observed across spatial extents (Fig. 3c). The increase in total explained variation from small to large spatial extents primarily reflected a stronger influence of spatially structured environmental variables at larger spatial extents (Fig. 3c, see Appendix S1). The influence of spatial variables also increased with spatial extent, but only weakly (Fig. 3c, see Appendix S1). Pure (spatially unstructured) environmental variables had a weak influence on ITV β-diversity at all spatial extents. In contrast, the increase in total explained variation from small to large spatial grains primarily reflected a stronger influence of pure environmental variables and a weaker influence of spatial variables (Fig 4c, see Appendix S1).

Environment and spatial influences on species-mean β-diversity were less strongly influenced by changes in spatial extent and uninfluenced or weakly influenced by changes in spatial grain. As with ITV β-diversity, the total amount of variation in species-mean β-diversity explained by environmental and spatial variables increased from the smallest spatial extent to the largest spatial extent (Fig. 3d, see Appendix S1). However, the overall magnitude of the increase was larger for ITV β-diversity (20% increase) compared to species-mean β-diversity (12% increase). Moreover, the total amount of variation in species-mean β-diversity explained by environment and space did not change from the smallest spatial grain to the largest spatial grain (Fig. 4d, see Appendix S1).

Environmental and spatial variables also had contrasting influences on taxonomic β-diversity at different spatial scales. The total amount of variation in taxonomic β-diversity explained by environment and space did not change between small and large spatial extents (Fig. 5c, see Appendix S1) but increased strongly between small and large spatial grains (Fig. 5d, see Appendix S1). As with both measures of functional β-diversity, taxonomic β-diversity was primarily influenced by spatially structured environmental variables at all spatial extents and grains. An exception was the largest spatial grain, at which pure environmental variables and spatially structured environmental variables had similar influences on taxonomic β-diversity.

Discussion

Our study illustrates the importance of considering how two different components of scale, spatial extent (variation in the

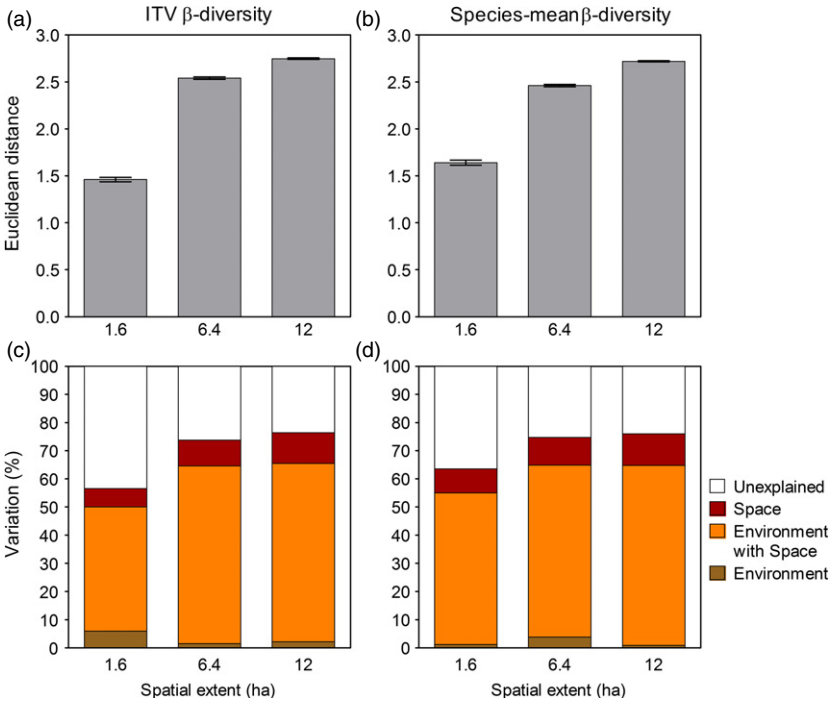

Fig. 3. Environmental and spatial influences on patterns of functional β-diversity at three spatial extents (1.6, 6.4 and 12 ha). (a) Patterns of functional β-diversity that include intraspecific trait variation (ITV) across habitat types (ITV β-diversity). (b) Patterns of functional β-diversity based on overall species-mean trait values (species-mean β-diversity). Each bar represents the average (±1 standard error) pairwise Euclidean distance of community weighted trait values among 20 × 20-m quadrats (n = 40, 160, and 299 quadrats for the three spatial extents, respectively). (c, d) Functional β-diversity explained by environmental and spatial variables. The four partitions show the adjusted R^2 values for environmental variables, spatially structured environmental variables, spatial variables and the unexplained variation based on distance-based redundancy analysis (see Appendix S1). Environmental variables are listed in Appendix S2.

overall sampling area with a fixed spatial grain) and spatial grain (variation in the area of a sampling unit within a fixed spatial extent), influence the contribution of ITV to patterns of functional β-diversity and inferred community assembly mechanisms across environmental gradients. We found that patterns of functional β-diversity that included ITV (ITV β-diversity), as well as the strength of their overall correlations with environmental and spatial gradients, varied more strongly with changes in spatial extent (Fig. 3a,c) than with changes in spatial grain (Fig. 4a,c). This result is consistent with the hypothesis that local-scale environmental filtering gives rise to locally adapted populations and high functional β-diversity. In contrast, patterns of functional β-diversity based on species-mean trait values (species-mean β-diversity) and their correlations with environment and space were less strongly influenced by changes in spatial extent (Fig. 3b,d) and uninfluenced or weakly influenced by changes in spatial grain (Fig. 4b,d). Together, these patterns suggest that the perceived importance of environmental filtering within spatially extensive landscapes depends on the degree to which ITV changes with spatial extent and spatial grain.

CHANGES IN ITV β-DIVERSITY ACROSS ENVIRONMENTAL GRADIENTS AND SPATIAL SCALES

We found that patterns of ITV β-diversity and their overall correlations with environmental and spatial variables increased from small to large spatial extents (Fig. 3, see Appendix S1). Spatially structured environmental variables explained more variation in ITV β-diversity than pure (spatially unstructured) environmental variables and spatial variables at all spatial extents (Fig. 3c, see Appendix S1). Moreover, the relative importance of spatially structured environmental variables increased from small to large extents. Given the high degree of spatial autocorrelation in the environment at this spatial grain (20 × 20 m), these patterns could reflect an increase in the relative importance of dispersal limitation, environmental filtering or both dispersal limitation and environmental filtering from small to large spatial extents (Gilbert & Lechowicz 2004; Legendre *et al.* 2009). Although our observational analyses alone cannot untangle these mechanisms across spatial extents, our results suggest that the perceived importance of environmental filtering increases with spatial grain (Fig. 4c). These results suggest an important influence of relatively fine-scale environmental filtering on patterns of functional β-diversity in this temperate forest, a finding that parallels results found at broader scales in other temperate (Siefert *et al.* 2013) and tropical (Swenson, Anglada-Cordero & Barone 2011) forests.

Patterns of ITV β-diversity and their correlations with environmental and spatial variables responded differently to changes in spatial grain (Fig. 4). In contrast to the pattern observed across spatial extents (Fig. 3a), ITV β-diversity decreased from small to large spatial grains (Fig. 4a). These contrasting patterns are likely explained by the influence of spatial grain on habitat associations of species across environmental gradients (Soininen *et al.* 2007; Morlon *et al.* 2008). For example, the amount of taxonomic β-diversity explained by pure environmental variables increased from small to large spatial grains (Fig. 5c), suggesting a more important role for species sorting among different habitat types at larger scales. As species sorting increases, we would expect less potential for local adaptation of populations among habitats as fewer species are distributed across habitat types, resulting in lower ITV β-diversity. Even so, more variation in ITV β-diversity was explained by pure environmental variables at large

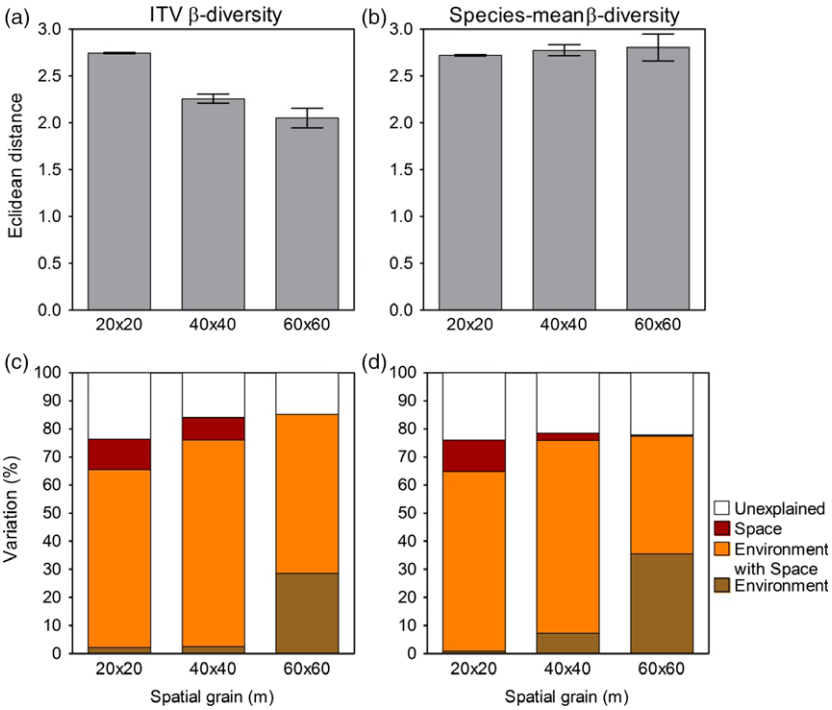

Fig. 4. Environmental and spatial influences on patterns of functional β-diversity at three spatial grain sizes (20 × 20 m, 40 × 40 m, 60 × 60 m). (a) Patterns of functional β-diversity that include intraspecific trait variation (ITV) across habitat types (ITV β-diversity). (b) Patterns of functional β-diversity based overall species-mean trait values (species-mean β-diversity). Each bar represents the average (±1 standard error) pairwise Euclidean distance of community weighted trait values among quadrats of a given grain size (n = 299, 66 and 28 quadrats for the three grain sizes, respectively). (c, d) Functional β-diversity explained by environmental and spatial variables. The four partitions show the adjusted R^2 values for environmental variables, spatially structured environmental variables, spatial variables and the unexplained variation based on distance-based redundancy analysis (see Appendix S1). Environmental variables are listed in Appendix S2.

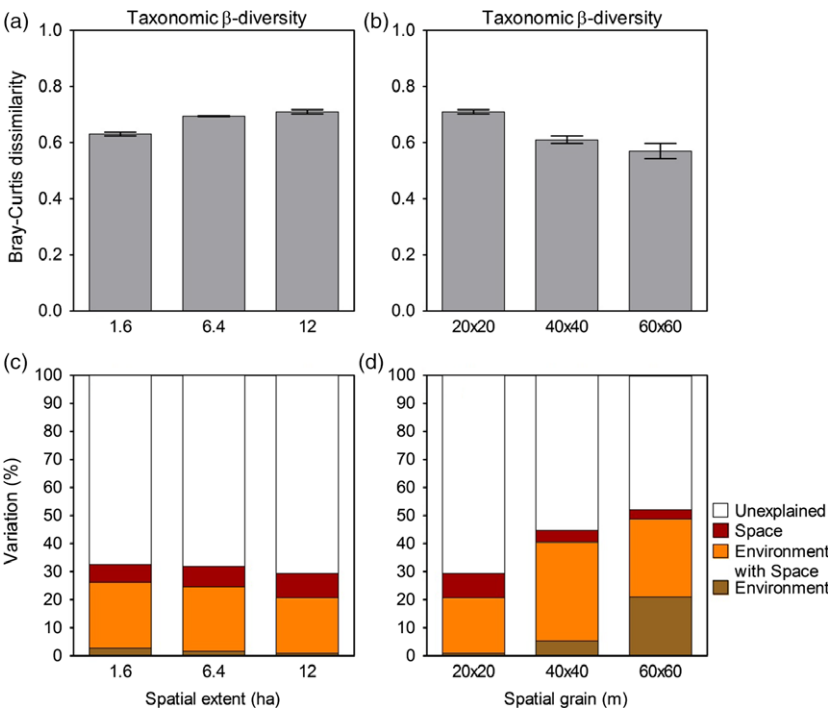

Fig. 5. Environmental and spatial influences on patterns of taxonomic β-diversity (mean Bray–Curtis dissimilarity ± 1 standard error) at three spatial extents (1.6, 6.4 and 12 ha; a and c) and three spatial grain sizes (20 × 20 m, 40 × 40 m, 60 × 60 m; b and d). The four partitions show the adjusted R^2 values for environmental variables, spatially structured environmental variables, spatial variables and the unexplained variation based on distance-based redundancy analysis (see Appendix S1). Environmental variables are listed in Appendix S2.

compared to small spatial grains, suggesting an increase in the relative importance of environmental filtering of locally adapted populations at larger spatial grains.

COMPARISONS BETWEEN ITV β-DIVERSITY AND SPECIES-MEAN β-DIVERSITY ACROSS SPATIAL SCALES

In contrast to previous studies that have largely inferred community assembly mechanisms based on species-mean trait values, our comparative approach allowed us to evaluate the extent to which ITV contributes to the perceived importance of environmental filtering. Overall patterns of ITV β-diversity were more strongly influenced by changes in both spatial extent and spatial grain compared to patterns of β-diversity based on species-mean trait values. Between the smallest and largest spatial extents, we observed a larger increase in the total amount of variation explained by environmental and spatial variables for ITV β-diversity (20% increase) compared to

species-mean β-diversity (12% increase) (Fig. 3c,d). This relatively modest increase in explained variation was likely influenced by the way in which we quantified ITV. Although our approach quantifies variation in mean trait values among habitats, it does not capture the entire range of ITV among all individuals in the landscape. Given that there is environmental variation within each habitat type that may contribute to local adaptation of traits, measuring traits on each individual would provide a more accurate (though logistically challenging) assessment of the contributions of ITV to functional β-diversity and the strength of environmental filtering. Nonetheless, the additional variance explained by ITV suggests that a combination of dispersal limitation and environmental filtering contribute to variation in functional traits among local populations within spatially extensive landscapes (e.g., Messier, McGill & Lechowicz 2010). Across spatial grains, in contrast, we observed an increase in the total amount of variation explained by environment and space for ITV β-diversity (10% increase; Fig. 4c), but little change in the explained variation for species-mean β-diversity (2% increase; Fig. 4d). Together, these results suggest that ITV contributes most strongly to patterns of functional β-diversity and inferred assembly mechanisms at spatial extents that maximize environmental heterogeneity, but spatial grains that minimize environmental heterogeneity at a given spatial extent (Fig. 1). If larger grain sizes capture more of the overall trait variation among populations, then ITV will contribute weakly to functional β-diversity, since all of the ITV for a given species will be clumped within one sampling grain.

COMPARISONS BETWEEN FUNCTIONAL β-DIVERSITY AND TAXONOMIC β-DIVERSITY

Our results underscore the importance of considering both taxonomic and functional diversity in studies of community assembly (McGill *et al.* 2006; Weiher *et al.* 2011). In general, we were able to explain less than half as much variation in taxonomic β-diversity as compared to either metric of functional β-diversity across the same environmental and spatial gradients (Figs 3–5). Although pure environmental variables and spatially structured environmental variables combined to explain more variation in taxonomic β-diversity than spatial variables, the majority of taxonomic β-diversity was unexplained, a pattern often interpreted as reflecting stochastic colonization and extinction processes that lead to ecological drift (Legendre *et al.* 2009). However, unexplained variation may also reflect important unmeasured abiotic variables (e.g., light, microclimate, micronutrients) or biotic interactions that were not considered in our study. In a previous study in this forest, we found higher functional diversity of leaf size and SLA than expected by chance among co-occurring saplings, suggesting a role for competition via limiting similarity (Spasojevic *et al.* 2014). Interestingly, taxonomic β-diversity increased from small to large spatial extents (similar to the patterns observed for functional β-diversity), but the total variation explained by environmental and spatial variables did not change with spatial extent (Fig. 5a,c). In contrast, the variation in taxonomic β-diversity explained by pure environmental variables increased with spatial grain (Fig. 5b,d). Together, these results suggest that species sorting across environmental gradients primarily occurs at scales larger than our smallest sampling grain (20 × 20 m). Similar patterns were found in another temperate forest and several tropical forests where a greater overall fraction of taxonomic β-diversity was explained with increasing spatial grain (Legendre *et al.* 2009; De Caceres *et al.* 2012).

Conclusions and future directions

Despite growing interest in the causes and consequences of both functional β-diversity and ITV, few studies have integrated these concepts explicitly when examining the relative importance of multiple assembly mechanisms (Swenson, Anglada-Cordero & Barone 2011). Our study provides a framework for further exploration of the ecological conditions under which ITV contributes to patterns of β-diversity across spatial scales. Specifically, our results suggest that studies of functional β-diversity need to be carefully designed and interpreted to account for ITV, spatial extent, and spatial grain. Sampling functional traits and the environment across scales which include both trait variation among species and trait variation among populations will likely increase our ability to infer the relative importance of community assembly mechanisms from patterns of functional β-diversity (Fig. 1).

Future studies on the linkages among ITV, environmental heterogeneity and scale will help to address several key challenges beyond the scope of the present study. First, it is important to recognize that changes in environmental filtering may vary among different ontogenetic stages within landscapes and among landscapes with different levels of environmental heterogeneity. In this temperate forest, for example, the strength of environmental filtering for some leaf traits may increase from the sapling to adult stage (Spasojevic *et al.* 2014). As our study focused only on understorey individuals (saplings and small-stature trees and shrubs), our results provide a relatively conservative estimate of the contribution of ITV to patterns of β-diversity across environmental gradients. In addition, our study site includes strong gradients of environmental heterogeneity that are strongly correlated with trait variation (e.g., >70% of the variation in functional β-diversity was explained by environmental and spatial variables at most scales; Figs 3 and 4). Comparative studies across a range of life-history stages and environmental gradients will help illuminate how ITV contributes to β-diversity and inferred mechanisms of community assembly. Thirdly, the relative contribution of ITV to patterns of functional β-diversity may vary systematically across larger-scale biogeographic gradients. For example, Hulshof *et al.* (2013) found greater ITV relative to interspecific trait variation in SLA at lower latitudes. To the extent that ITV increases towards the tropics (Hulshof *et al.* 2013), then ITV may have a much stronger influence on patterns of functional β-diversity across spatial scales (grain and extent) in tropical relative to temperate ecosystems. Although studies to date have not explicitly

compared how ITV contributes to functional β-diversity across temperate and tropical ecosystems, a recent study in tropical forests suggested an important influence of ITV on patterns of β-diversity (Swenson, Anglada-Cordero & Barone 2011). Comparative studies of ITV across biogeographic gradients will provide new insights into the ecological conditions under which trait variation within and among species contributes most strongly to patterns of biodiversity across spatial scales.

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Author contributions

MJS and JAM conceived the study; MJS and JAM collected the field data; BLT analysed the soil samples; MJS analysed the data and wrote the first draft of the paper; all authors contributed to revisions.

Data accessibility

Plant composition and environmental data available at the CTFS-ForestGEO data-access portal: http://ctfs.si.edu/Public/plotdataaccess/. Trait data available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.rr4pm (Spasojevic & Myers 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The relative contribution of intraspecific trait variation and interspecific trait variation to the total trait variation within each habitat type for each trait.

Appendix S1. Results from a distance-based redundancy analysis used to partition variation functional β-diversity that includes ITV among habitat types (ITV β-diversity), functional β-diversity that excludes ITV (species-mean β-diversity), and taxonomic β-diversity (Bray Curtis dissimilarity) into fractions explained by environmental and spatial variables at three spatial extents (1.6, 6.4 and 12 ha) and three spatial grains (20 × 20 m, 40 × 40 m, 60 × 60 m).

Appendix S2. Significance of environmental and spatial variables used in the distance-based redundancy analyses of β-diversity at three spatial extents and spatial grains.

Appendix S3. Trait data for all 681 measured individuals.