

Disentangling dispersal from phylogeny in the colonization capacity of forest understorey plants

Lander Baeten^{1,2*}, T. Jonathan Davies³, Kris Verheyen¹, Hans Van Calster⁴ and Mark Vellend⁵

¹Department of Forest and Water Management, Ghent University, Geraardsbergensesteenweg 267, 9090 Gontrode, Belgium; ²Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium; ³Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal QC H3A 1B1, Canada; ⁴Research Institute for Nature and Forest (INBO), Kliniekstraat 25, 1070 Brussels, Belgium; and ⁵Département de Biologie, Université de Sherbrooke, 2500 Boulevard de l'Université, Sherbrooke QC J1K 2R1, Canada

Summary

1. Habitat patches that have been completely cleared of their original vegetation historically and subsequently recolonized naturally provide a useful study system to explore the importance of the processes involved in community assembly. Forests where the understorey vegetation is recovering from past agricultural land use form an iconic example of such a system.
2. The colonization capacity of forest plant species into post-agricultural forests has been related to dispersal traits in previous comparative analyses, demonstrating the significance of dispersal limitation. Yet, none of them has evaluated evidence for a phylogenetic signal in colonization capacity and, thus, explored the possibility that the dispersal traits are correlated with unmeasured establishment-related traits that are also shared through common ancestry.
3. Here, we analysed the colonization capacity of 330 species into post-agricultural forests across seven different landscapes in Europe and North America. With phylogenetic meta-analysis models, we quantified the phylogenetic signal in colonization capacity and tested whether the colonization – dispersal trait relationships are confounded by phylogenetic non-independence.
4. Closely related forest understorey species were more similar to one another in terms of their capacity to colonize post-agricultural forests than were more distantly related species. The correlations between dispersal traits and colonization were not independent from phylogeny. While we found some evidence of phylogenetic clustering of species' frequencies in post-agricultural communities, this was apparently not a result of strong filtering on dispersal traits.
5. *Synthesis.* Given the phylogenetic signal in plant colonization capacity, a multitude of conserved species characteristics may explain community assembly in forests. Earlier trait-based syntheses strongly emphasised dispersal, but the factors limiting establishment and persistence of forest herbs in post-agricultural forests may be more nuanced than generally appreciated.

Key-words: ancient forest, determinants of plant community diversity and structure, dispersal limitation, forest herbs, land-use change, life-history traits, meta-analysis, phylogenetic signal, secondary succession

Introduction

Dispersal is a fundamental process in community ecology determining the distributions and abundances of plant species' populations (Vellend 2010). Direct evidence for dispersal-limited distributions comes from seed sowing or transplantation experiments (Turnbull, Crawley & Rees 2000; Clark *et al.* 2007), although such experiments typically involve only

short-term monitoring (often only seedling emergence) and just one or a few species. Comparative analyses exploring relationships between the real-world distribution patterns of species and their life-history traits related to dispersal thus serve as a complementary line of evidence to experiments. Landscapes where many habitat patches have been completely cleared of their original vegetation historically, and subsequently recolonized naturally, provide a useful study system in this context (e.g. Cramer, Hobbs & Standish 2008). Community assembly takes place via dispersal from remnant populations into the recovering patches and, species' colonization

*Correspondence author: E-mail: Lander.Baeten@UGent.be

success can be evaluated against dispersal traits to explore the role of dispersal limitation.

An iconic example of such a system is the many forested landscapes in western Europe and north-eastern America where there is a history of forest clearance for agriculture and subsequent spontaneous recovery of the understorey vegetation over several decades (Flinn & Vellend 2005; Hermy & Verheyen 2007). Seed sowing experiments in recovering ‘post-agricultural forests’ initially showed several understorey plant species to be principally dispersal limited (Graae, Hansen & Sunde 2004; Verheyen & Hermy 2004), but long-term monitoring beyond the seedling stage showed post-dispersal ‘establishment limitation’ to be at least equally important (Baeten, Hermy & Verheyen 2009a; Baeten *et al.* 2009b). Screening of life-history traits has shown that the species which fail to occupy post-agricultural forests after several decades, relative to their presences in historically uncleared ‘ancient forests’, are characterized by traits associated with low dispersability in space and time. Species with low colonization capacity, the so-called ancient forest plant species, typically have heavy seeds, low seed release height and short-distance dispersal syndromes (e.g. ant or unassisted dispersal) (Graae & Sunde 2000; Verheyen *et al.* 2003b; De Frenne *et al.* 2011). These findings suggest that dispersal limitation might shape the process of community assembly in forest understorey communities. However, such analyses do not allow one to rule out the possibility that dispersal traits are simply correlated with unmeasured traits related to the ability of species to establish and persist in post-agricultural forests, the latter of which might be additional causes of interspecific variation in colonization capacity.

By controlling for shared phylogenetic history between species – and therefore additional traits shared through common ancestry – phylogenetic comparative methods permit stronger tests of potential causal links between different species’ characteristics (Harvey & Pagel 1991). None of the studies testing for trait–colonization relationships in temperate forest herbs has taken account of the phylogenetic non-independence among species. Discerning the ecological importance of dispersal traits of understorey plant species requires a phylogenetic analysis if traits determining colonization capacity are phylogenetically conserved, that is when related species share similar traits that influence their colonisation capacity. A complementary approach to assessing the processes underlying community assembly is to test for clustering (i.e. underdispersion) or overdispersion of trait or phylogenetic differences among species occupying the same habitat (Webb *et al.* 2002). In the case of post-agricultural forest colonization, the hypothesis of strong dispersal limitation would predict underdispersion of dispersal traits in post-agricultural forests. To the extent that dispersal traits are evolutionarily conserved, this hypothesis would also predict phylogenetic underdispersion.

Here, we reanalyse seven data sets from Europe and north-eastern America, which were used previously to test the role of dispersal traits in determining the colonization capacity of understorey forest plants (Verheyen *et al.* 2003b). We focus

specifically on life-history traits related to effective species’ seed dispersal distances (Thomson *et al.* 2011; Tamme *et al.* 2014): seed mass, seed release height and dispersal syndrome. First, we analysed the relation between colonization capacity (i.e. relative species frequencies in post-agricultural vs. ancient forests) and the traits in a multi-response phylogenetic meta-analysis model (Hadfield & Nakagawa 2010; Nakagawa & Santos 2012), which improves upon the earlier analyses in several important ways. The model incorporates phylogenetic dependency, accounts for the uncertainty associated with the estimate of colonization capacity (the measurement error commonly used in meta-analyses) and considers both the colonization capacity and the dispersal traits as responses (accounting for phylogenetic non-independence in both). Secondly, we also analysed species frequencies (i.e. the proportion of sites where present) in ancient or post-agricultural forest, exploring the phylogenetic clustering of these frequencies and the clustering of traits. If the recolonization of post-agricultural forests is strongly filtered on dispersal traits, we expect higher trait similarity for species in post-agricultural relative to ancient forest communities (i.e. clustering of traits). For evolutionarily conserved traits, this would also cause stronger phylogenetic clustering of species frequencies in the post-agricultural forest communities (Webb *et al.* 2002). With this combination of analyses, we tested several new ecological questions: (i) Is there a phylogenetic signal in the colonization capacity of forest understorey plant species? (ii) What is the relative importance of common ancestry in generating relationships between the traits related to seed dispersal distance and colonization capacity? (iii) Do the relationships between colonization capacity and seed mass and seed release height vary between dispersal syndromes? (iv) Are species frequencies phylogenetically clustered and is this a result of filtering on dispersal traits?

Materials and methods

DATA SETS

We used the data base from an earlier comprehensive meta-analysis that quantified the relationship between forest plant colonization rates and a broad set of life-history traits, including seed mass, seed release height and dispersal syndrome (Verheyen *et al.* 2003b). This data base comprised 12 north-western European and eight north-eastern American data sets. Each data set was a list of species and their relative frequency in ancient and post-agricultural forest, estimated from a census of forest patches in a landscape where the species composition of a patch was assessed either by walk-throughs or by random vegetation plots (range 150–500 m²). We selected data sets from this original data base as follows. First, several data sets sampled only few ancient or post-agricultural forest patches (< 15) and were excluded because the relative species frequencies calculated from few patches or plots are an unreliable estimate of their probability of presence within the studied landscape. Note that the earlier meta-analysis was less sensitive to sample size because colonization patterns were pooled across studies to calculate a single species-level metric of colonization rate – in this study, we specifically retain the within-species variation across data sets. Secondly, some data sets included only a subset of the understorey community, for example only reporting

species significantly associated to either ancient or post-agricultural forest or using fixed species checklists. Only data sets comprising complete understorey communities were retained, again to enable unbiased modelling of the between- and within-species variation in colonization capacity. Thirdly, while the American data sets were all collected in plots, six of the European data sets were species lists from entire forest patches of highly variable size (e.g. from 1 to > 100 ha in central Lincolnshire, UK; Peterken & Game 1984), which may confound estimates of the probability of presence of species. Exclusion of the patch-based data sets was already achieved after application of the previous criteria.

The data sets we retained were (i) Brunet (1994) – Sweden, $N = 354$ plots, 85 understorey plant species; (ii) Hermy (1985) – Belgium, $N = 396$, 166 species; (iii) Sciama *et al.* (2009) – France, $N = 105$, 112 species; (iv) Gerhardt (1993) – Massachusetts, USA, $N = 56$, 33 species; (v) Glitzenstein *et al.* (1990) – New York, USA, $N = 76$, 27 species; (vi) Singleton *et al.* (2001) – New York, USA, $N = 50$, 27 species; (vii) Sobey (1995) – Prince Edward Island, Canada, $N = 1200$, 69 species. More detailed information about the data sets is provided in table 1 in Verheyen *et al.* (2003b). The post-agricultural forests in these studies were all established between 60 and 170 years before sampling, with no consistent difference in time since agricultural abandonment between continents. The original data base included understorey herbs (forbs and graminoids) as well as shrubs, trees, mosses and vines. Here, we focus on the understorey herbs only. We restrict our focus for two reasons. First, especially in Europe, shrub and tree species are generally planted or sown shortly after agricultural abandonment and therefore do not undergo spontaneous colonization. Secondly, in an analysis combining trees, shrubs and understorey plants, a trait such as seed release height would be almost perfectly confounded with growth form. The total number of data lines (i.e. unique understorey species \times site combination) was 514 for a total of 330 unique species (233 species in Europe and 109 in North America).

SPECIES TRAITS AND PHYLOGENETIC TREE

We focussed on three life-history traits directly related to seed dispersal distance: seed mass, maximum seed release height and dispersal syndrome (Thomson *et al.* 2011). We updated or filled gaps in the trait data used in Verheyen *et al.* (2003b) if more comprehensive data sources were available. Seed mass for European species was updated using the average generative dispersule mass from the LEDA traitbase (Kleyer *et al.* 2008). For North America, we filled gaps using the Kew Seed Information Database (Royal Botanic Gardens Kew 2014). Seed mass data (mg) were available for 71% of the species. The LEDA traitbase also provided seed release height (m) data for the European species (generally equal to plant height), while for the North American species, we retained the plant height (m) used in Verheyen *et al.* (2003b). Plant height is a good proxy of seed release height: both traits were strongly correlated for the European understorey herbs (trait data from LEDA; $N = 204$, $r = 0.86$, $P < 0.001$). Trait coverage of seed release height was 82%. Finally, the dispersal syndrome of European species was taken from the Ecological Flora of the British Isles (Fitter & Peat 1994), and gaps were filled using the Kew data base for North American species (62% coverage). The dispersal syndrome categories were ant, animal (bird or mammal), explosive, unspecialized and wind. For purposes of the analyses, we grouped syndromes to reflect adaptations for long-distance dispersal (animal, wind) and short-distance dispersal (ant, explosive, unspecialized). This simplification is reasonable because the syndromes them-

selves are already quite broad categories reflecting combinations of particular dispersal characteristics and because many species exhibited multiple syndromes (i.e. they could not be assigned to single syndromes). If species had adaptations for long- and short-distance dispersal (e.g. both animal and explosive), we assigned it to the long-distance category (only 21 species) given the overwhelming importance of rare long-distance dispersal for colonization (e.g. Nathan *et al.* 2008).

We constructed a phylogenetic tree for the complete data set using the online tool Phylomatic (Webb & Donoghue 2005), in which a taxon list is matched against a backbone ‘metatree’, returning a pruned tree of genus-level relationships. A recent phylogenetic hypothesis of the Angiosperm Phylogeny Group was used as backbone tree (R20120829 for plants). Then, branch lengths were scaled proportional to time based on known node ages from Wikström, Savolainen & Chase (2001) using the BLADJ algorithm in the PHYLO-COM software (Webb, Ackerly & Kembel 2008). To test the sensitivity of our results to the phylogenetic tree used, we extracted a second tree for the European species using the comprehensive dated supertree ‘DaPhnE’, which is also based on the backbone family phylogeny of the Angiosperm Phylogeny Group, but which is dated using recent molecular studies (Durka & Michalski 2012). The ‘DaPhnE tree’ represented 98% of the European species we analysed.

MULTI-RESPONSE PHYLOGENETIC META-ANALYSIS

The response of a species to land-use change can be expressed as a response ratio, assuming that the presences of a species in ancient (A) and post-agricultural (AG) forest come from two independent binomial distributions, for example $y_A \sim B(n_A, p_A)$ where n_A and p_A are the number of sampled plots and the probabilities of presence in ancient forest, respectively. The proportion of the plots in which a species occurs in post-agricultural and ancient forest is an estimate of p , and their ratio $R = \bar{p}_{AG}/\bar{p}_A$ is an effect size measuring the species’ response to land-use change (De Frenne *et al.* 2011). This effect size equals the commonly used ‘rate ratio’ metric calculated for 2×2 contingency tables (in this case species presence/absence in two land-use history categories) and is generally expressed as the natural logarithm $\ln R = \ln(R)$. The metric $\ln R$ is approximately normally distributed with measurement (or sampling) error variance $\sigma_v^2 = (1 - \bar{p}_A)/(n_A \bar{p}_A) + (1 - \bar{p}_{AG})/(n_{AG} \bar{p}_{AG})$ (Hedges, Gurevitch & Curtis 1999; Rosenberg, Rothstein & Gurevitch 2013), and is further referred to as the ‘colonization capacity’ of a species in a data set: $\ln R < 0$ indicates low colonization capacity (typical ancient forest species), and $\ln R > 0$ indicates strong association with post-agricultural forests and therefore better colonization capacity. We excluded rare species with estimated probabilities of presence in both ancient and recent forest < 5% from the analyses, because these estimates might be unreliable due to factors such as low detection probability.

We first fitted univariate multilevel models with colonization capacity as the response, continent ('C'; to account for the almost completely different species pools) and one of the three dispersal traits ('TR') as predictors and species as a grouping variable (random intercepts). These models provide a reference analysis not including phylogenetic dependencies. A single model with the three dispersal traits was fitted as well, but this model required complete data lines for the three traits together, which reduced the total sample size. The model can be written as: $\ln R_i = \mu_i + s_{k[i]} + \varepsilon_i$ and $\mu_i = \beta_0 + \beta_1 C_i + \beta_2 TR_{k[i]}$, where β_0 is the intercept, β_1 and β_2 are regression coefficients, and $TR_{k[i]}$ is a species-level value for one of the three dispersal traits ($k = 1, \dots, N_{species}$) (notation following

Gelman & Hill 2007; Nakagawa & Santos 2012). The species-level errors $s \sim N(\mathbf{0}, \sigma_s^2 I)$ have a between-species variance σ_s^2 , and the residuals $\varepsilon \sim N(\mathbf{0}, \sigma_e^2 I)$ have residual (within species) variance σ_e^2 . Two versions of the model were fitted, one without and one with an additional measurement error effect $\mathbf{m} \sim N(\mathbf{0}, \mathbf{M})$, where \mathbf{M} is a diagonal matrix with the known measurement error variances for each species in each study (σ_v^2 – see above) as elements. The model with measurement error weights data points by variance and is a meta-analytic model. We used Bayesian analyses of generalized linear multi-level models implemented in the *MCMCglmm* function in R 3.0.2 (Hadfield 2010; R Core Team 2013). For the random species effect, we used a commonly applied inverse Wishart prior (parameters $V = 1$ and $nu = 0.002$, with V the expected variance if the degree of belief parameter nu goes to infinity). We ran three independent chains to check model convergence. Each chain was set to 100 000 iterations, after a burn-in of 5000, and a thinning interval of 100 (resulting in 1000 samples). We report posterior modes $\pm 80\%$ and 95% highest posterior density intervals of the first chain in the results.

A phylogenetic meta-analysis model was constructed by extending the previous meta-analytic model, thus including the measurement error effect, with an additional phylogenetic effect (Hadfield & Nakagawa 2010; Nakagawa & Santos 2012): $\mathbf{a} \sim N(\mathbf{0}, \sigma_a^2 \mathbf{A})$, where \mathbf{A} is a $N_{\text{species}} \times N_{\text{species}}$ correlation matrix of evolutionary distances between species extracted from the phylogenetic tree and σ_a^2 the phylogenetic variance. In this model, the previous species-level variance term σ_s^2 is then the between-species variance independent of the phylogeny. A high phylogenetic variance indicates trait differences among species are largely proportional to their phylogenetic relatedness, while large differences between close relatives returns a high estimate of σ_s^2 (high between-species variance independent of phylogeny). A metric of the phylogenetic signal equivalent to Pagel's λ is $H^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_s^2 + \sigma_e^2)$ (Nakagawa & Santos 2012). When $H^2 = 0$, this is equivalent to no phylogenetic signal and $H^2 = 1$ means trait variation between species is perfectly consistent with Brownian motion evolution down the phylogeny.

We considered the colonization capacity as well as the dispersal traits as response variables, to account for the phylogenetic non-independence in both and to estimate the phylogenetic signal for both. Therefore, we extended the phylogenetic meta-analysis model to its multivariate form (Hadfield & Nakagawa 2010). In this multi-response model, the variance components that are estimated (i.e. σ_a^2 , σ_s^2 and σ_e^2) are replaced by their multivariate analogues. For instance, \mathbf{V}_a is a 2×2 (co)variance matrix with the phylogenetic variances for the colonization capacity and a dispersal trait on the diagonal and the covariance between these phylogenetic effects as off-diagonal elements. In this study, we are particularly interested in the relative importance of the correlation between colonization capacity and the dispersal traits (calculated from the covariance and variances) based on the phylogenetic effects (effects a) vs. the correlation based on the species-level effects independent of the phylogeny (effects s). Since the colonization capacity and dispersal traits were estimated for different individuals, the residual covariance was set to zero. Measurement errors for the dispersal traits were unknown and were set to one (i.e. no weights applied). Models were fitted using *MCMCglmm* as above. For the random effects, we used an inverse Wishart prior again (parameters: V equal to the 2×2 identity matrix and $nu = 1.002$). The dispersal syndromes were lumped into short- vs. long-distance dispersers (see above), and we did not directly test for a phylogenetic signal between these relatively heterogeneous groups. However, we analysed the colonization capacity–seed mass relationship and the colonization capacity–seed release height relationship for the entire data set and within the different dispersal syndrome groups separately.

CLUSTERING OF TRAITS AND SPECIES FREQUENCIES

Following from the hypothesis that dispersal traits determine colonization capacity, we predicted that recolonized post-agricultural communities represent a non-random subset of the species in the phylogeny, filtered on dispersal ability. To test this prediction, we calculated the phylogenetic clustering of species frequencies and the clustering of the continuous dispersal traits (seed mass, seed release height) in ancient vs. post-agricultural communities. For phylogenetic clustering, the abundance phylogenetic deviation ‘APD’ metric can quantify whether the more frequently sampled species in a data set are more related than expected ($APD > 0$) (Hardy 2008). When $APD < 0$, the more frequent species are spread among distantly related clades (overdispersion). Null models were used to test whether the APD differed from zero – the significance was calculated as the proportion of null communities with equal or larger APD than observed (equal or smaller for negative APD). We used a null model reshuffling the species that were observed in at least one site among the tips of the phylogeny (see Hardy 2008). APD was calculated for the ancient (APD_A) and post-agricultural (APD_{AG}) forest sites for each data set and compared with a paired *t*-test (alternative hypothesis $APD_A < APD_{AG}$, i.e. phylogenetic clustering is stronger in post-agricultural sites).

If community assembly is strongly filtered on dispersal traits, the most frequent species should have similar dispersal trait values (relatively low variance). The weighted (by species frequency) average trait values and the variance on these weighted averages were calculated to compare trait values and trait clustering for ancient vs. post-agricultural forest. A generalized least squares (GLS) model with the trait as response, land-use history as predictor and 1/relative frequency as covariate in a fixed variance function exactly quantifies such weighted averages and variances. Trait data were log-transformed to remove the mean–variance relationship, allowing independent comparisons of weighted averages and variances. For each data set, we compared two nested alternatives of the GLS model with a likelihood ratio test either assuming equal or different variances for ancient and post-agricultural forests. No difference indicates that the trait dispersion is independent of land-use history.

Results

Seed mass was significantly negatively related to colonization capacity in the univariate models without phylogenetic correction, that is heavy seeded species had lower colonization capacity (slope $\beta_2 = -0.05$) (Fig. 1). Higher seed release height and long-distance dispersal syndromes were related to better colonization (positive slopes of 0.14 and 0.26, respectively). This result was relatively robust to the inclusion of measurement error, although the effects were somewhat lower and seed release height and dispersal vector became only marginally significant (95% credible intervals or CI's just included zero) (black symbols Fig. 1). Estimating the effects of the three dispersal traits within a single model, that is only for the species with complete data for the three traits ($N = 108$ species), returned similar results for seed mass (slope = -0.07 , 95% CI $[-0.11, -0.02]$) and dispersal vector (slope = 0.21 , 95% CI $[-0.02, 0.43]$), whereas the effect of seed release height was reduced (slope = 0.03 , 95% CI $[-0.08, 0.18]$). The effect of continent was marginally significant and

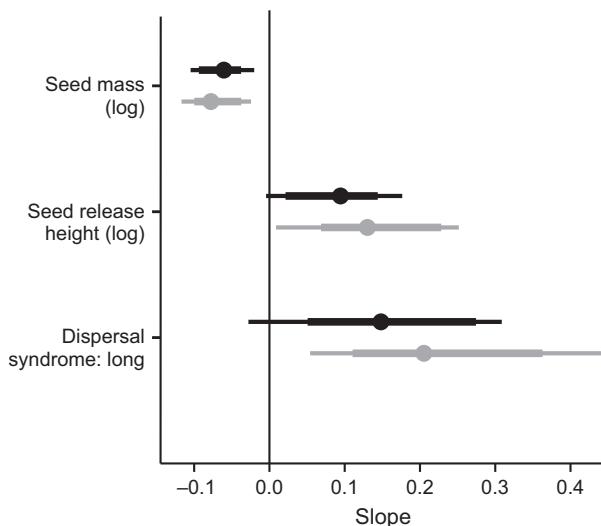


Fig. 1. Results of univariate multilevel models with the colonization capacity as response and one of three dispersal traits as predictor variables. The estimated slopes $\pm 80\%$ (thick lines) and 95% (thin lines) credible intervals are shown for models that account (black) or do not account (grey) for measurement error. The slope for dispersal syndrome shows the effect of long- vs. short-distance dispersal syndromes on species' colonization capacity. Data from three European and four American studies were used, for a total of 151 (seed mass), 186 (release height) and 149 species (dispersal syndrome).

indicated that the American data sets contained on average more species with low colonization capacity (effect $\beta_1 = -0.26$, 95% CI $[-0.47, 0.03]$).

Closely related species tended to have similar colonization capacities (Figs 2 and S1 in Supporting Information), and the phylogenetic signal for colonization capacity was consistent across the multi-response models, with estimates of $H^2 = 0.43$ and 0.44 (Table 1). Seed mass showed very strong phylogenetic conservatism ($H^2 = 0.95$), and the phylogenetic signal for seed release height was intermediate ($H^2 = 0.65$). These results were robust to the choice of phylogenetic topology; for the European species, very similar levels of phylogenetic conservatism were found for colonization capacity, seed mass and seed release height using either the Phylomatic or DaPhnE topology (Table S2).

Independent of phylogeny, the relationship between the seed mass and colonization capacity was small and not different from zero (Fig. 3a). The negative correlation between seed mass and colonization based on the phylogenetic effects was significant, indicating that the negative relationship observed in the non-phylogenetic models above emerged because of shared evolutionary history. Similar patterns emerged within the group of species that have adaptations for long-distance dispersal (i.e. wind or animal dispersed). For species with short-distance dispersal syndromes (ant, explosive, unspecialized), the correlations based on the phylogenetic effects and the correlations based on the phylogeny-independent species effects were both smaller and not different from zero (Fig. 3a). Results were similar using the DaPhnE phylogeny (Fig. S3). For seed release height, the positive rela-

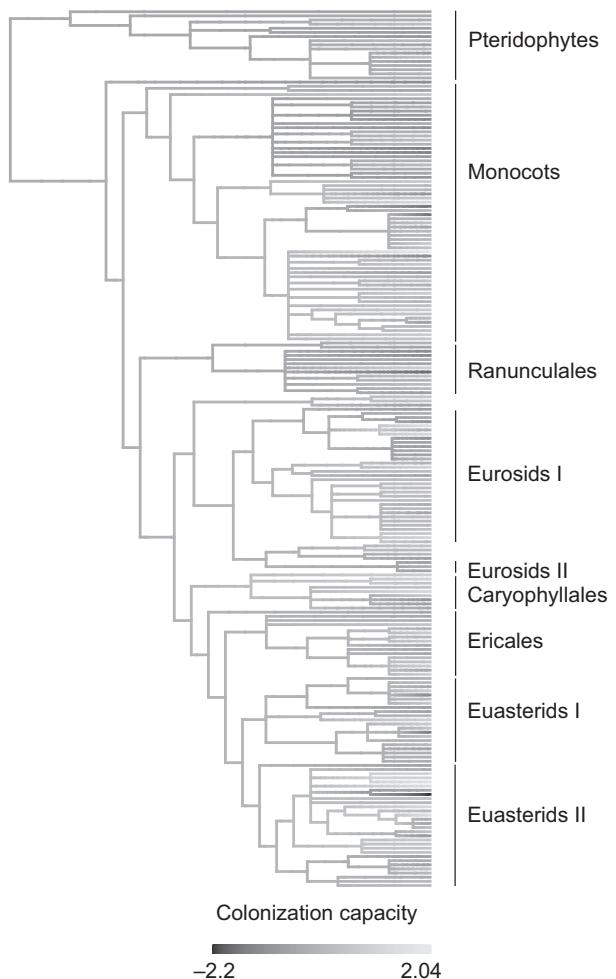


Fig. 2. Phylogenetic tree (topology from Phylomatic) with the distribution of the species' colonization capacity from low (black) to high (light grey). The mapping of the colonization capacity is based on maximum-likelihood estimation of states at internal nodes and interpolation of the states along each edge (*contMap* in the *phytools* R package; Revell 2013). This graph is mainly intended for visualisation, not to actually quantify the ancestral states. The species shown come from the three European and four American data sets – the alternative topology from DaPhnE for the European data only is provided in Fig. S1.

tion found in the univariate, non-phylogenetic models could not be attributed to the phylogenetic relationships between species only: the correlations between release height and colonization capacity based on the phylogenetic effects as well as the correlations independent of the phylogeny were small and not significantly different from zero (Fig. 3b). The positive seed release height–colonization capacity pattern is thus explained in part by phylogenetic effects and in part by species-specific effects independent of phylogeny and neither is significant on its own. Distinguishing between short- and long-distance dispersal syndromes did not change the conclusions.

The phylogenetic clustering of species frequencies was higher in post-agricultural compared to ancient sites in five out of seven data sets (paired *t*-test $t = -1.55$, $P = 0.086$)

Table 1. Phylogenetic signal expressed as the phylogenetic heritability metric H^2 (\pm 95% credible intervals)

Model responses	N_{species}	Colonization capacity		Dispersal trait	
		H^2	95% CI	H^2	95% CI
Seed mass, colonization capacity	151	0.43	[0.26, 0.62]	0.95	[0.84, 0.99]
Release height, colonization capacity	186	0.44	[0.26, 0.61]	0.65	[0.38, 0.82]

The metric was calculated for two multi-response models, each with colonization capacity and either seed mass (log-transformed) or seed release height (log-transformed) as the response variable. Here, we used the Phylomatic phylogenetic topology and all seven European and American data sets (see Table S1 for DaPhnE phylogenetic topology).

(Table 2). In two data sets, the most frequent species in post-agricultural forest tended to be more related than expected ($\text{APD} > 0$, $P < 0.1$). For ancient forest, one data set showed significant clustering and one showed overdispersion. In none of the data sets was the trait variance different between ancient and post-agricultural forest for seed mass or seed release height (Table 2). A comparison across data sets showed that the weighted average seed mass was significantly higher in ancient compared to post-agricultural forest (ancient = 2.77 mg and post-agricultural = 2.41 mg; paired t -test on log-transformed mass $t = 2.56$, $P = 0.042$), but the variances were not different (paired $t = 0.94$, $P = 0.38$). For the weighted average seed release height, we found no difference between land-use history types (ancient = 37 cm and post-agricultural = 38 cm; paired t -test on log-transformed height $t = -0.86$, $P = 0.423$), and the variances were also not different (paired $t = 1.53$, $P = 0.18$).

Discussion

We found a clear phylogenetic signal in colonization capacity: closely related forest understorey species are more similar to one another in terms of their capacity to colonize post-agricultural forests than are more distantly related species. This implies that land-use change not only influences plant species composition and diversity as well as genetic variation within species in post-agricultural forests (Flinn & Vellend 2005; Hermy & Verheyen 2007; Vellend *et al.* 2007), but also the phylogenetic structure of the understorey communities. Clades such as *Liliales*, *Ericales* and *Ranunculales*, for instance, typically show lower presences in post-agricultural forests relative to ancient forest in temperate Europe and North America. Since phylogenetic affinities are generally reflected in species' functional similarities (Webb *et al.* 2002), an underrepresentation (or overrepresentation) of particular clades can have important ecological consequences. A more practical implication of the phylogenetic conservatism of colonization capacity is that it would be possible to impute expected recovery rates for species that were not yet studied in the context of land-use change (e.g. in north-eastern Asian temperate forest), based on their evolutionary relationship with species from the well-documented regions in western Europe and north-eastern North America (Garland & Ives 2000).

Following on from many non-phylogenetic studies (Graae & Sunde 2000; Verheyen *et al.* 2003b; De Frenne *et al.* 2011), we reanalysed the relationships between colonization capacity and key traits related to seed dispersal distance (Thomson *et al.* 2011; Tamme *et al.* 2014), to explore the role of common ancestry in generating apparent patterns of dispersal limitation. The negative correlation between seed mass and colonization (better colonizers are small-seeded species) is not independent from phylogeny, and the reduction in correlation when correcting for phylogeny was most important for species that are animal or wind dispersed. Likewise, the positive relation between colonization capacity and release

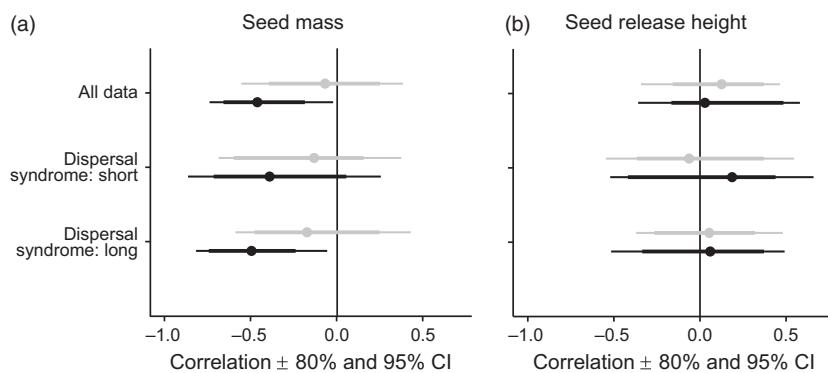


Fig. 3. Results of multi-response models with the colonization capacity and either (a) log-transformed seed mass or (b) log-transformed seed release height as response variables, using the European and American data sets and the Phylomatic phylogenetic topology. Estimated correlations between colonization capacity and the dispersal traits come in two forms: correlations based on phylogenetic effects showing relationships emerging because of shared evolutionary history (black) and correlations independent of the phylogeny (grey). Lines show the 80% (thick lines) and 95% (thin lines) credible intervals. Models were fitted using the entire data set ('All data') or using the subset of species with long-distance (wind, animal dispersal) or short-distance dispersal syndromes (ant, explosive, unspecialized).

Table 2. Phylogenetic clustering of species frequencies (APD metric) and trait dispersion (variance σ^2 on weighted average) in ancient (A) vs. post-agricultural (AG) forest sites across the seven data sets

Study	Phylogenetic clustering		Seed mass (log)			Seed release height (log)		
	APD _A	APD _{AG}	σ_A^2	σ_{AG}^2	P	σ_A^2	σ_{AG}^2	P
1. Sweden	-0.009	-0.016	0.90	0.92	0.92	0.06	0.06	0.78
2. Belgium	0.015	0.052	0.32	0.29	0.50	0.10	0.08	0.57
3. France	0.020	0.021	0.86	0.74	0.47	0.08	0.09	0.84
4. MA, USA	0.039	0.014	1.51	1.32	0.77	0.35	0.34	0.94
5. NY, USA	-0.045	0.047	1.04	1.30	0.74	0.13	0.14	0.94
6. NY, USA	-0.024	-0.007	1.64	1.25	0.58	0.19	0.12	0.23
7. PEI, Canada	-0.032	0.011	0.27	0.27	0.95	0.07	0.04	0.21

Bold APD values are significantly different from zero ($P < 0.1$). Trait variances were estimated using GLS models assuming different variances for the two land-use histories; differences in variance were tested in a comparison with nested GLS models assuming equal or unequal variances (likelihood ratio test P value).

height (better colonizers are generally taller) was also confounded by phylogenetic non-independence, that is closely related species with similar colonization rates also resembled each other in terms of seed mass and seed release height. This observation is consistent with studies showing that cross-species relationships between dispersal distance, and these traits emerge largely through few divergences in deep phylogenetic nodes (Thomson *et al.* 2011).

There are two not mutually exclusive interpretations considering the actual importance of dispersal limitation in post-agricultural community assembly. First, dispersal really is the significant bottleneck for the successful recovery of forest herb communities after land-use change, but we do not have sufficient independent evolutionary origins to detect its significance in a phylogenetic analysis. Some complementary lines of evidence support the importance of dispersal, such as the relationships of species distributions with the spatiotemporal isolation from colonization sources (Peterken & Game 1984; Brunet & Von Oheimb 1998; Singleton *et al.* 2001; Verheyen *et al.* 2003a) and the initial establishment success after experimental introductions (Graae, Hansen & Sunde 2004; Verheyen & Hermy 2004). Secondly, unmeasured traits related to establishment and persistence in post-agricultural forests may be additional causes of interspecific variation in colonization capacity, and dispersal is simply correlated to these traits via shared ancestry. For example, recovery of plant communities is often constrained by altered interactions with a disturbed and degraded soil community (Kardol & Wardle 2010), and related species may be similar in their dependency on plant mutualists and susceptibility to plant enemies of such modified soil biota, though this remains an open question (Flinn & Vellend 2005). The soil nutrient availability and stoichiometry in post-agricultural forests also often bear the imprint of the past fertilization (e.g. leading to low N : P-ratios; Baeten *et al.* 2011), which relates to traits involved in the competition for light and leaf economy traits, but, at the same time, also relates to the investment in sexual reproduction (Fujita *et al.* 2013). Establishment and dispersal traits are thus correlated across species and may simultaneously determine their

response to the nutrient legacies of past land use. Finally, tree species composition and density is generally also influenced by land-use history, and by their profound effect on the light and soil nutrients, tree identity effects on the understorey might be relevant as well. Supporting evidence in this context comes from few demographic studies that have found important effects of abiotic and biotic (plant-plant interactions) land-use legacies on particular post-seedling stages of plant establishment (Endels *et al.* 2004; Vellend 2005; Baeten *et al.* 2009b). In sum, while there is clear evidence for dispersal-limited community assembly in post-agricultural forests, our results indicated that the previous trait-based syntheses may have led to an overemphasis on the dominant role of dispersal. We show, therefore, that focussing on individual traits, here dispersal traits, without consideration of phylogenetic relatedness can mislead inference on the processes important to community assembly because such traits likely covary with other ecologically relevant traits through descent. In addition, we note that traits should be considered in the context of a plant's entire life-history (e.g., the advantage of small seed size for dispersal is offset by disadvantages in post-dispersal stages) and that 'dispersal traits' (especially seed size) may capture multiple aspects of a plants ecological strategy, not only dispersal distance (see Moles & Westoby 2006).

In addition to the cross-species comparative analyses, we also tested for evidence of phylogenetic clustering of species frequencies in the post-agricultural communities and explored whether this could be explained by strong filtering on the dispersal traits, previously called 'selective dispersal assembly' (Vellend *et al.* 2007). While we found some evidence of phylogenetic clustering of species frequencies, this was apparently not a result of strong filtering on seed mass or seed release height. It should be noted, however, that this hypothesis deserves further exploration with community data at the local scale, instead of the landscape-wide species frequencies we used here, as these data might not characterize actual species co-occurrences within plots or forest patches. Nonetheless, our study is the first to look at the effect of land-use change on interspecific trait variances in temperate forest communities

and, results suggest that the dispersion of trait values related to seed dispersal is not different in post-agricultural vs. ancient forest. Future studies might additionally benefit from looking at intraspecific trait variability as well, given that forest plant species differ significantly in their phenotypic plasticity in response to land-use change (Fraterrigo, Turner & Pearson 2005; Baeten *et al.* 2011); and the ecological filtering in community assembly acts upon this real-world variation between individuals, not the species-level trait means (Viole *et al.* 2012). For example, species with comparatively low seed release height in ancient forest may, for instance, actually grow very tall in post-agricultural forests where they benefit from soil nutrient legacies of the past fertilization (e.g. *Geum urbanum*, *Urtica dioica*; Baeten *et al.* 2011). Direct trait measurements in forests with contrasting history are, however, currently not available for most species.

In sum, while our study might not be definitive with respect to the relative importance of dispersal limitation for community assembly following land-use change, it does indicate that the conclusion that dispersal limitation is the dominant control on post-agricultural forest community assembly should be revisited. Earlier syntheses (using similar data) strongly emphasised dispersal, but the factors limiting establishment of forest herbs in post-agricultural forests may be more nuanced than generally appreciated. Given the phylogenetic signal in the colonization capacity, a multitude of other conserved species characteristics may explain differences in colonization success. Important directions for future research include more elaborate analyses of trait vs. species clustering and large scale field measurements of traits, in addition to more widespread application of long-term introduction experiments.

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Data accessibility

All the data used in our meta-analyses are publicly available in the original publications and the online trait data bases and phylogenies.

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Supporting Information

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

Figure S1. Phylogenetic tree (DaPhnE topology) with distribution of colonization capacity for the European data.

Figure S2. Results of multi-response models based on the DaPhnE phylogeny.

Table S1. Phylogenetic signal H^2 based on the Phylomatic vs. DaPhnE phylogeny.