

TOWARD PREDICTION IN THE RESTORATION OF BIODIVERSITY

Restored tallgrass prairies have reduced phylogenetic diversity compared with remnants

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Summary

1. Ecological restoration is critical for mitigating habitat loss and providing ecosystem services. However, restorations often have lower diversity than remnant, reference sites. Phylogenetic diversity is an important component of biodiversity and ecosystem function that has only recently been used to evaluate restoration outcomes. To move towards prediction in the restoration of biodiversity, it is necessary to understand how phylogenetic diversity of restorations compares with that of reference sites, and where deficits are found, to evaluate factors constraining phylogenetic diversity.

2. We quantified plant taxonomic and phylogenetic diversity in eastern tallgrass prairie, one of the most endangered ecosystems on earth. We measured diversity at large (site) and small (plot) scales in 19 restored prairies and compared patterns with those from 41 remnant prairies. To evaluate how environmental conditions and management actions influence outcomes, we tested the effects of soil properties and seed mix composition on diversity of restorations.

3. Restored prairies were less phylogenetically diverse than remnants at both spatial scales. On the other hand, the total species richness of remnant and restored prairies did not significantly differ, but remnants had higher native richness. Restored communities were taxonomically and phylogenetically distinct from remnants.

4. Soil properties (moisture and pH) influenced phylogenetic diversity and composition. There were positive relationships between the taxonomic and phylogenetic diversity of seed mixes and resulting diversity of planted assemblages (excluding volunteer species). Species in seed mixes were more closely related than expected by chance, and several clades found in remnant prairies were missing from seed mixes.

5. *Synthesis and applications.* Restored tallgrass prairies had lower phylogenetic diversity than remnant prairies, which may contribute to the widely observed phenomenon of restorations not being functionally equivalent to reference sites. It is encouraging for restoration efforts that seed mix phylogenetic diversity predicted phylogenetic diversity of planted assemblages. This indicates that designing phylogenetically diverse seed mixes for restoration is beneficial. In addition, clades found in reference sites that are missing from restoration seed mixes could be added to new or existing restorations to reduce gaps in phylogenetic diversity. Further work on the effects of management on phylogenetic diversity is needed to advance restoration of biodiversity.

Key-words: biodiversity, composition, establishment, grassland, phylogenetic diversity, phylogeny, reference site, seed mix, soil, tallgrass prairie

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Introduction

Ecological restoration is essential for biodiversity conservation and ecosystem service provision in a changing world (Rey Benayas *et al.* 2009; Possingham, Bode & Klein 2015). Phylogenetic measures of biodiversity – metrics that reflect evolutionary distance among species in a community – are predictive of ecosystem functions and properties that are important restoration objectives (Srivastava *et al.* 2012; Hipp *et al.* 2015). Plant communities with higher phylogenetic diversity have been shown to be more productive, stable, diverse at higher trophic levels and resistant to invasion than those with lower phylogenetic diversity (Cadotte, Cardinale & Oakley 2008; Davies, Cavender-Bares & Deacon 2011; Cadotte, Dinnage & Tilman 2012; Dinnage *et al.* 2012; Li *et al.* 2015).

Further, phylogenetically informed measures of biodiversity can be more predictive of ecosystem function than other diversity metrics, such as species richness (SR) and functional diversity (e.g., Cadotte *et al.* 2009). The relationship between phylogenetic diversity and ecosystem function is partly driven by phylogenetic information providing a proxy for functional trait information via phylogenetic niche conservatism (Wiens & Graham 2005). Phylogenetic information can also account for ecologically relevant variation not captured by measured traits alone (Cadotte *et al.* 2009; Pearse & Hipp 2009; Larkin *et al.* 2015a). Because phylogenetic diversity is often closely related to ecosystem function, phylogenetic analyses can inform ecological restoration (Verdu, Gomez-Aparicio & Valiente-Banuet 2012; Hipp *et al.* 2015; Barber *et al.* 2016).

Beyond their use in predicting ecosystem function of restored sites, phylogenetic measures can enrich understanding of remnant communities, relatively undegraded sites that can serve as reference sites for restoration (Larkin *et al.* 2015a). Phylogenetic measures of biodiversity can combine aspects of SR, composition and evolutionary history and help to provide a deeper understanding of reference communities. Comparing phylogenetic diversity and composition of reference and restored sites can pinpoint deficits that may be correctable through management actions (e.g., adding species from clades that are represented in remnants, but absent from restorations). Understanding discrepancies between restored and reference systems with respect to phylogenetic diversity and composition can also aid in identifying historical constraints to restoration and shaping future restoration objectives (Barak *et al.* 2016; Turley & Brudvig 2016). Whilst there are many metrics for quantifying phylogenetic diversity, mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) are commonly used in community ecology and have shown utility for community-level comparisons (Tucker *et al.* 2016). MPD and MNTD are less confounded by SR than other phylogenetic diversity metrics, and therefore are useful in comparative studies with SR. We henceforth refer to

MPD and MNTD measures jointly as ‘phylogenetic diversity.’

Phylogenetic diversity is a potentially useful criterion for designing functional restorations, particularly if restoration inputs (e.g., seed mixes) are reliable predictors of outcomes. Plant community inputs comprise the potential restored community, but relationships between the composition of seed mixes and properties of resulting plant communities are not always strong. Seeded species need to pass through multiple filters to be represented in restorations: emerging, establishing and persisting only with the right confluence of species biology, management actions and site conditions (Hillhouse & Zedler 2011; Grman & Brudvig 2014; Grman *et al.* 2015; Barber *et al.* 2016). Because of this, many seeded species fail to become part of realized plant communities (Foster *et al.* 2007; Hillhouse & Zedler 2011; Grman *et al.* 2015). Soil properties can also constrain the restoration of biodiversity and ecosystem function (Baer, Heneghan & Eviner 2012; Grman & Brudvig 2014; Sollenberger *et al.* 2016). To advance restoration of phylogenetic diversity, it is critical to understand how seed mixes and site conditions shape restoration outcomes.

We evaluated taxonomic and phylogenetic components of plant diversity in restored tallgrass prairies and compared patterns with those found in remnant prairies. We had several goals: First, we tested the hypotheses that restorations would have lower taxonomic and phylogenetic diversity than remnants, and that restorations and remnants would differ in taxonomic and phylogenetic community composition. Since differences between remnant and restored sites are often scale-dependent, we assessed these relationships at two spatial scales (plot and site) (Allison 2002; Martin, Moloney & Wilsey 2005). Second, we tested factors that may influence restorations’ phylogenetic diversity outcomes, evaluating the influence of soil properties and seed mix composition on resulting assemblages. Lastly, we sought to identify phylogenetic deficits in restored prairies that could be remedied through management actions, by identifying ‘missing branches’ – clades that were either absent from seed mixes, or that were present in mixes, but that failed to establish or persist in restored prairies.

Materials and methods

SITE DESCRIPTIONS

We surveyed 19 restored prairies within 11 preserves in north-eastern Illinois, USA. Restorations were initiated between 1998 and 2012 (mean: 8.3 ± 3.5 SD years restored) and ranged in size from 0.7 to 300 ha (mean: 27.0 ± 66.3 SD, See Table S1, Supporting Information). All restorations were initiated or at some point managed by the same restoration contractor (Pizzo and Associates, Leland, IL, USA). We obtained seeding lists for all sites from restoration managers. We were unable to consistently obtain records for other management actions that might be influential, e.g., burning and/or herbicide treatment,

and seeding rate. Such factors are thus not included in this analysis.

We used plant community data from 41 remnant prairies as a reference dataset for evaluating restorations. These sites include some of the highest quality remnant prairies in the region; they exhibit no evidence of soil disruption (e.g., due to past tillage agriculture) but are embedded within a largely developed/agricultural landscape (Bowles & Jones 2013). While all remnants and restorations are in northeastern Illinois, they are not within the same preserves and therefore did not share substrates or management regimes. Remnant prairies were surveyed by Bowles & Jones (2013). Detailed phylogenetic and functional trait analyses of these remnants are reported in Larkin *et al.* (2015a). Though Bowles & Jones (2013) and Larkin *et al.* (2015a) report data from both 1976 and 2001, we used only 2001 data in our comparisons with restorations. We surveyed restored prairies using the same methods as Bowles and Jones to facilitate comparisons.

VEGETATION SURVEYS

At each study site, we placed two 50-m transects. Study plots (0.25 m² round quadrats) were positioned every 5 m along each transect, for a total of 20 plots per site. Each plot was randomly placed either left or right of the transect line and offset by a randomly selected distance of 2.0–7.0 m (at 1.0-m intervals). We identified all taxa within each plot to species, or genus if we could not reliably identify to species. Samples that could not be identified in the field were collected and pressed for identification at the Chicago Botanic Garden. Vegetation surveys were conducted between 16 June and 4 August 2015. Later in the season (September 2015), we returned to each site to conduct a directed search for species present at the site, but not captured in our transect survey data. Two researchers (EWW and RSB) walked through each site for at least 60 min, identifying and recording species. For comparisons between restored and remnant prairies, we define 'site scale' diversity as the additive richness across all 20 plots at each site. For comparisons of restored sites and their seed mixes, 'site scale' also included additional species found in our directed search (which was not performed during sampling of remnants).

Taxonomic names for all species found in remnants, restorations and seed mixes were standardized using the Taxonomic Name Resolution Service (Boyle *et al.* 2013). Species were assigned native status according to Swink & Wilhelm (1994) and the USDA PLANTS database (USDA 2016).

SOIL DATA

For 18 of the 19 restored sites, we collected and composited two 15-cm deep soil cores from within each surveyed plot (we were not permitted to collect soil from the remaining site). For each sample, we measured gravimetric soil moisture (GSM), soil pH, organic matter by mass loss on ignition (LOI) and electrical conductivity (EC). Samples were sifted through a metal sieve with 0.5-cm openings to remove rocks, roots and other debris. Sub-samples (4–10 g) were weighed, dried in an oven to constant mass (≥ 24 h) at a temperature of 105 °C and weighed again to calculate GSM. To measure LOI, dried samples were placed in a muffle furnace at 300 °C for 4 h, moved to a 105 °C oven for 20 min and then to a desiccator for 30 min, and weighed. We tested pH and EC using a handheld probe (Multi-parameter PCSTestr 35;

Eutech/Oakton Instruments, Vernon Hills, IL, USA) on dried soil rehydrated with approximately 15 mL of deionized water. We calibrated pH values using standard solutions of pH 4.01, 7.00 and 10.01 and EC values using 1413 and 12880 $\mu\text{S cm}^{-1}$ solutions. As GSM and LOI values were highly collinear, we used only GSM values in our analyses. GSM and EC values were natural-log transformed to better approximate normality for statistical analyses.

PHYLOGENETIC TREE

We constructed a community phylogeny of 589 species found in restored and reference sites and seeding lists by modifying a published tree of 32 223 plant taxa (Zanne *et al.* 2014). We grafted taxa not present in the Zanne *et al.* tree (131 identified to species, 63 identified to genus), and pruned taxa absent from our dataset in R version 3.1.3 (R Development Core Team 2013), using the `weldTaxa` and `make.matAndTree` functions in the `Morton` R project (A. Hipp, <https://github.com/andrew-hipp/morton>). The Zanne *et al.* tree was constructed using GenBank sequences for seven gene regions (18S rDNA, 26S rDNA, ITS, matK, rbcL, atpB and trnL-F) using maximum likelihood for tree estimation. A single non-angiosperm species found in a restored site (*Sceptridium multifidum*) was excluded from analysis.

DATA ANALYSES

All analyses were performed using R version 3.1.3. We calculated three measures of biodiversity – SR and two measures of phylogenetic diversity, MPD and MNTD – at both plot and site scales. MPD is the mean phylogenetic distance between all pairs of species in a community; MNTD is the mean phylogenetic distance between each species and its closest relative in the community. Metrics were calculated based on the presence-absence of species in plots and sites, i.e., were not abundance-weighted (Webb *et al.* 2002; Kembel *et al.* 2010).

To remove the influence of SR on MPD and MNTD, we calculated standard effect sizes: (observed value – expected value)/standard deviation of the expected value. We calculated expected values under a 'frequency' null model with 999 permutations using the functions `SES.MPD` and `SES.MNTD` in the `picante` package (Kembel *et al.* 2010). The frequency null randomizes the presence/absence of species across samples while controlling for species' frequency of occurrence. Positive values of `SES.MPD/SES.MNTD` indicate that species present in the community are more distantly related than expected by chance (phylogenetically overdispersed), while negative values indicate that species are more closely related than expected by chance (clustered). Consequently, higher values of `SES.MPD/SES.MNTD` indicate higher phylogenetic diversity.

We tested for differences in diversity (SR, `SES.MPD` and `SES.MNTD`) between remnant and restored sites. At the site scale, we tested for significant differences using ANOVA; at the plot-scale we used linear mixed-effects models, with site as a random effect, to account for non-independence of plots within sites. We repeated analyses with all species included and with only native species included (to reflect plant assemblages targeted in restoration). Mixed-effects models were fit using the `lme` function in the `nlme` package (Pinheiro *et al.* 2016).

We used linear models to test the effects of prairie size and years since restoration was initiated (age) on each diversity metric

at the site scale, and mean plot scale. We natural-log transformed area to better approximate a normal distribution. To test for effects of soil properties (GSM, pH and EC) on diversity measures, we used linear models at the site scale and linear mixed-effects models at the plot scale, with site as a source of random error. For the linear mixed-effects models we report both marginal and conditional R^2 as described in Nakagawa & Schielzeth (2013).

We also investigated the relationships between restored prairies and the seed mixes used to establish them. To determine whether seed mix diversity was predictive of site diversity, we constructed linear models with seed mix diversity as the predictor variable and site diversity as the response variable. We separately tested the effects of seed mix diversity on site diversity of all species in communities and of only the planted community, i.e., the subset of species seeded into each site, excluding colonizing 'volunteer' species.

We tested for differences in community composition between remnants and restorations and between restorations and seed mixes, using site-level presence-absence data. To compare the taxonomic composition between groups, we performed non-metric multidimensional scaling ordination using the *vegan* package (Oksanen *et al.* 2016). We also performed community phylogenetic ordinations wherein dissimilarity was based on phylogenetic, rather than taxonomic, distance between samples, using the functions *comdist* (for MPD-based calculations) and *comdistnt* (for MNTD) in *picante*. We tested for significant differences between community types (remnants vs. restorations, seed mixes vs. sites) using permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) with the *adonis* function in *vegan*. We evaluated which species were driving differences between groups by quantifying the contributions of each species to overall Bray–Curtis dissimilarity using the similarity percentage (*SIMPER*, Clarke 1993) function in *vegan*.

We also evaluated the effects of environmental factors on community composition with redundancy analysis (*rda*, Legendre & Legendre 2012) for continuous predictors using the *capscale* function in *vegan*. We used site-level mean values of GSM, EC and pH as predictor variables and site-level community composition as response variables. We tested for significance using the ANOVA function, with 999 permutations, and calculated R^2 values using the function *RSquareAdj* in *vegan*.

Lastly, we tested whether species included in restoration seed mixes, and species that emerged and persisted after planting, were more closely related to one another than expected under a null model. We first tested for phylogenetic signal in planted species, relative to the full phylogeny of all species present in remnants and restorations. The characteristic evaluated was binary, i.e., whether or not a given species was planted. To test this, we calculated the D statistic (Fritz and Purvis 2010) using the *phylo.d* function in *caper* (Orme *et al.* 2013). We estimated D and tested whether it was phylogenetically random ($D = 1$), non-random ($D < 1$), or structured as would be expected under a Brownian motion model of evolution ($D = 0$). We also tested for phylogenetic signal in two continuous characteristics: planting frequency (proportion of seed mixes in which a particular species occurred) and persistence (proportion of sites a given planted species was found in during sampling). We evaluated these by calculating the K statistic using the *phylosignal* function in *picante*. $K = 1$ indicates the degree of phylogenetic signal expected under Brownian motion, while $K < 1$ and $K > 1$

indicate lower and greater than expected phylogenetic signal respectively (Blomberg, Garland & Ives 2003). For both D and K , significance was evaluated by comparing observed values with results from 999 permutations of tip-shuffling randomizations. Phylogenetic signal of persistence was separately tested for all seeded species and for more commonly seeded species (included in seed mixes for ≥ 3 sites).

Results

REMNANT VS. RESTORED PRAIRIES

We found a total of 341 species in restored sites representing 54 families. Of these, 252 species from 42 families were found during transect surveys. An additional 89 species, and 12 families were found through the directed search. In contrast, there were 353 species from 64 families found at remnant sites (Table S2).

Restored prairies had lower phylogenetic diversity than remnant prairies by all metrics and at both spatial scales (Fig. 1, Appendix S1). On average, MPD of remnant prairies was 17 million years higher (raw MPD, 261.5 ± 1.5 vs. 244.5 ± 2.5 , 1 SE), and MNTD was 19 million years higher in remnants (71.6 ± 1.6 vs. 52.6 ± 1.5) at the site scale (Appendix S1). SES.MPD and SES.MNTD values indicated that restorations, but not remnants, were phylogenetically clustered (Fig. 1). The same patterns persisted when non-native species were excluded from analyses (Appendix S1). SR did not significantly differ between remnant and restored prairies at site or plot scales when all species were included. However, when non-native species were excluded, mean SR was higher in remnant prairies at both scales.

Remnant and restored communities were distinct from one another in taxonomic and phylogenetic composition (Fig. 2). *SIMPER* analyses indicated that taxonomic differences were driven primarily by more frequent occurrence of regionally common native species occurring as volunteers in restored prairies (e.g., *Ambrosia artemisiifolia*, *Oxalis stricta*, *Symphotrichum pilosum*, *Oenothera biennis*, Table S3). Secondly, these differences were associated with higher frequency of common restoration species in restored sites (e.g., *Penstemon digitalis* and *Bouteloua curtipendula*) and habitat specialist, conservative species in remnant sites that were not found in restorations (e.g., the hemiparasite *Comandra umbellata*, Swink & Wilhem 1994).

FACTORS INFLUENCING DIVERSITY OF RESTORED SITES

Restoration size and age were not significant predictors of plot or site scale diversity (Table S4). At the site scale, soil moisture (GSM) was a significant predictor of SES.MNTD ($F_{1,16} = 2.243$, $R^2 = 0.234$, $P = 0.042$). However, this trend was strongly influenced by a single site with very high soil moisture and was not significant with

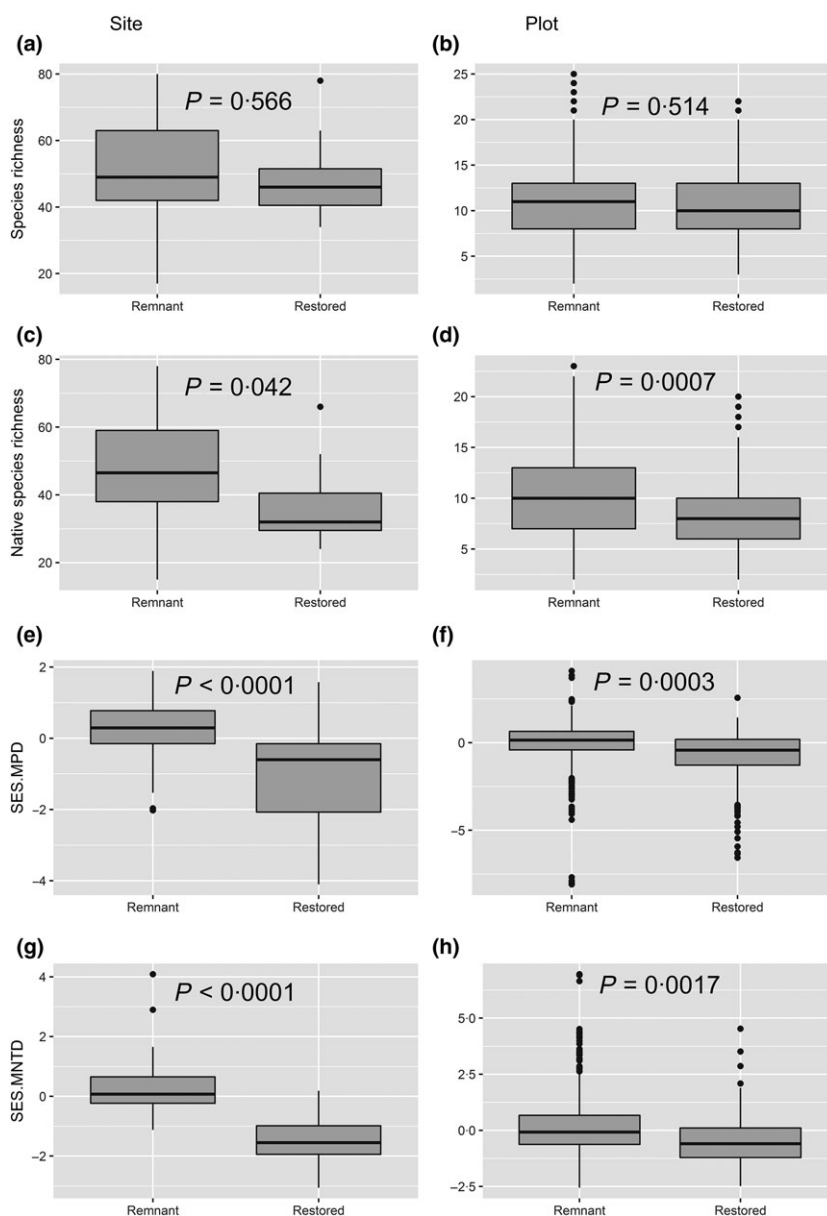


Fig. 1. Site and plot diversity metrics for species richness (a, b), species richness with non-native species excluded (c, d) and standard effect sizes for mean pairwise distance (MPD) (e, f) and mean nearest taxon distance (MNTD) (g, h). Boxplot centres represent median values and hinges the 25th and 75th percentiles. F values (all d.f. 1,58): (a) 0.332, (b) 0.431, (c) 4.385, (d) 7.728, (e) 22.556, (f) 15.106, (g) 39.194, (h) 10.827.

that site excluded. In plot-scale analyses, soil moisture was a significant predictor of SES.MNTD ($F_{1,339} = 8.00$, conditional $R^2 = 0.177$, marginal $R^2 = 0.042$, $P = 0.005$), even when the outlier site was excluded ($F_{1,320} = 4.72$, conditional $R^2 = 0.157$, marginal $R^2 = 0.024$, $P = 0.030$). In multivariate analyses (rda), pH was a significant predictor of taxonomic community composition, and pH and soil moisture were significant predictors of phylogenetic community composition (MNTD, Table S5).

Restoration seed mixes included a total of 209 species. Seed mix richness ranged from 29 to 125 species (mean ± 1 SE = 63.3 ± 6.8). The proportion of planted species observed at each site (including both transect surveys and directed searches) ranged from 25% to 77% ($44.6 \pm 3.4\%$). Seed mix diversity was not a significant predictor of overall plant community diversity but was significant when volunteer species were excluded (Fig. 3).

Equations from raw MPD and MNTD data are as follows, SR: $y = 0.28x + 10.35$, MPD: $y = 1.35x - 98.38$, MNTD: $y = 0.86x + 12.77$). Equations for raw values are reported for ease of interpretation (raw and SES values were highly correlated, $R^2 = 0.999$ for both MPD and MNTD).

Restored plant communities were taxonomically and phylogenetically distinct from seed mixes when all species were included and, to a lesser degree, when only planted species were included (Fig. 2). When all species were included, seed mix and site differences were driven by the presence of non-native species or volunteer native species present in many restorations, but few or no seed mixes (Fig. 2d). When only planted species were included, restored communities showed much more overlap with seed mixes, despite loss of seeded species that failed to establish (Fig. 2g).

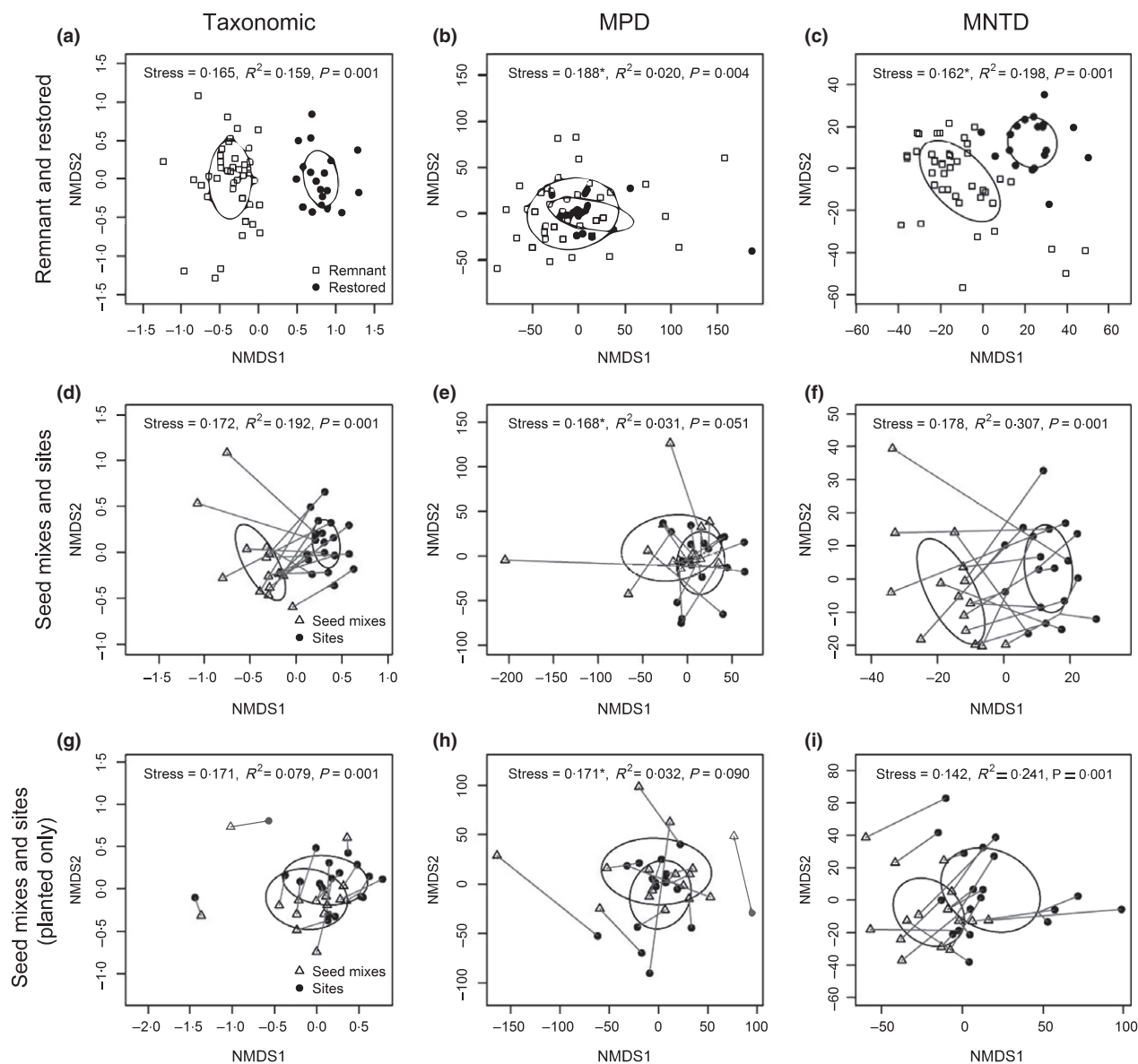


Fig. 2. Non-metric dimensional scaling ordinations of remnant and restored prairies and seed mixes, based on community taxonomic (a, d, g) and phylogenetic ordinations: mean pairwise distance (MPD) (b, e, h) and mean nearest taxon distance (MNTD) (c, f, i). In d-i lines connect each restored community to their planted seed mix. Number of dimensions $K = 2$ unless indicated by '*', then $K = 3$. Ellipses represent one standard deviation.

We found significant phylogenetic signal with respect to which species were seeded ($D = 0.65$, $P < 0.001$ relative to random null, Fig. 4). Several families well-represented in remnant prairies were not present in any seed mixes; these included the Liliaceae, Orchidaceae, Convolvulaceae and Polygalaceae. The entire order Caryophyllales (Amaranthaceae, Cactaceae, Caryophyllaceae, Phytolaccaceae, Polemoniaceae, Polygonaceae, Portulacaceae and Primulaceae families) was absent from seed mixes. Of the species that were planted, there was no phylogenetic signal in the proportion of seed mixes each species appeared in ($K = 0.003$, $P = 0.82$) or in their persistence ($K = 0.005$, $P = 0.42$). When we repeated these tests using only species seeded in at least three sites, the same pattern emerged ($K = 0.005$, $P = 0.49$ and $K = 0.005$, $P = 0.42$).

Discussion

PHYLOGENETIC DIVERSITY OF RESTORED VS. REMNANT PRAIRIES

Restorations exhibited consistently lower phylogenetic diversity than remnants regardless of the metric used or whether all or only native species were included. In addition, restorations' native SR and phylogenetic diversity was lower than that of remnants at both site and plot scales; this contrasts with previous findings using non-phylogenetic metrics that restorations may reach reference levels of diversity for one but not multiple spatial scales (Allison 2002; Martin, Moloney & Wilsey 2005; Middleton, Bever & Schultz 2010). Understanding the processes

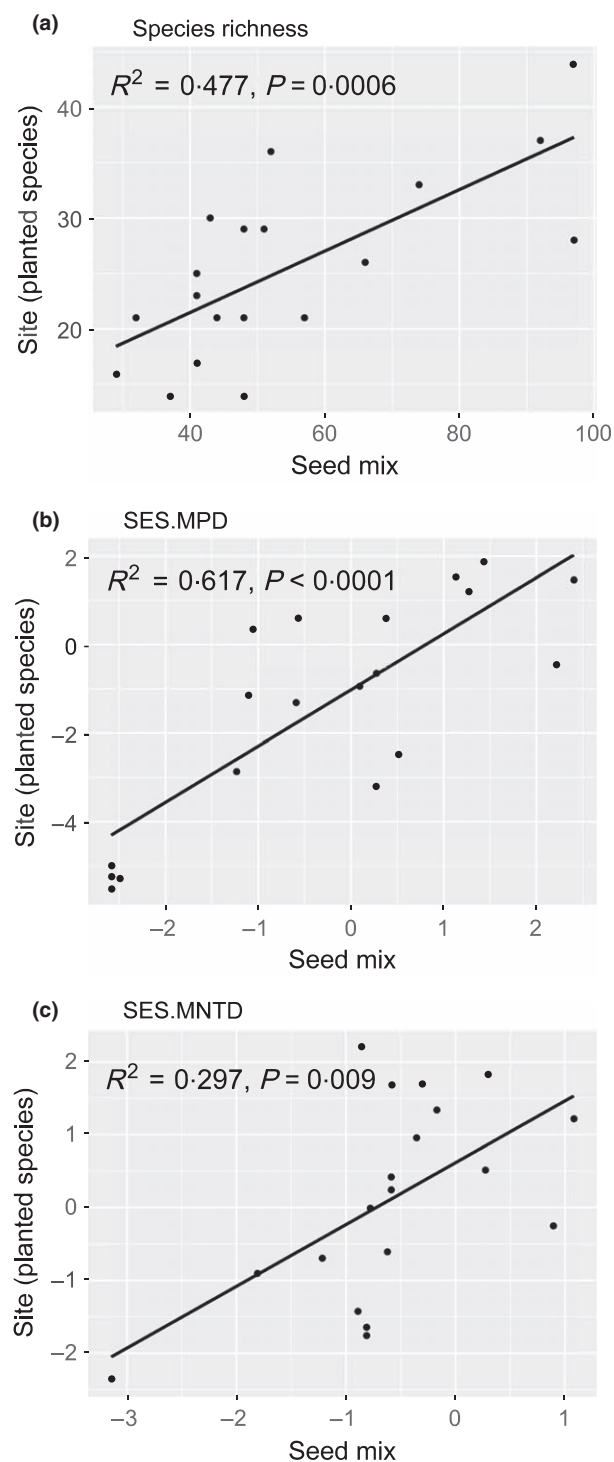


Fig. 3. Taxonomic and phylogenetic diversity of seed mixes and resultant prairie communities for only species planted at each site, for species richness (a), standard effect sizes of mean pairwise distance (b) and mean nearest taxon distance (c). Results from linear regression. Values for all species (not shown, species richness (SR): $R^2 = -0.055, P = 0.817$, SES.MPD: $R^2 = -0.040, P = 0.590$, SES.MNTD: $R^2 = 0.087, P = 0.118$).

that mediate diversity at different scales is an important step towards restoring ecosystems that are predictably more similar to remnants (Polley, Derner & Wilsey 2005).

The finding that restored prairies had lower phylogenetic diversity than remnants is in agreement with a small number of studies that have tested for differences between relatively intact natural habitats and degraded or disturbed habitats. For example, successional old fields disturbed by mowing showed lower than expected phylogenetic diversity (Dinnage 2009). Turley & Brudvig (2016) found persistent negative effects of agricultural legacies on phylogenetic diversity in longleaf pine savannas. Loss of historical disturbance regimes has also been linked to lower phylogenetic diversity, as in the case of unburned remnant prairies (Larkin *et al.* 2015a). The finding that disturbed sites tend to harbour communities that are more closely related than expected by chance may reflect harsh environmental conditions acting as a filter on community membership (Dinnage 2009). In the restored prairies we studied, such effects would be compounded by the restoration inputs themselves (i.e., seed mixes) having been phylogenetically clustered.

FACTORS DRIVING PHYLOGENETIC DIVERSITY IN RESTORED PRAIRIES

In order to advance prediction in the restoration of biodiversity, it is necessary to not only compare restorations to reference communities, but also to interpret variation between existing restorations (Brudvig *et al.*, 2017). Among the restored prairies, soil moisture and pH were drivers of diversity and composition. Soil properties have been linked to plant phylogenetic diversity, and soil moisture to establishment of species from seed mixes (Grman & Brudvig 2014; Sollenberger *et al.* 2016). However, effects of anthropogenic disturbance on contemporary habitats may overwhelm underlying environmental gradients, such as those imparted by soil heterogeneity (Alstad *et al.* 2016).

In sampled restorations, seed mix diversity was predictive of planted assemblages' SR and phylogenetic diversity. Use of more phylogenetically diverse seed mixes yielded greater phylogenetic diversity despite, on average, <50% persistence of seeded species. Given the relationship between phylogenetic diversity and ecosystem function, these results may indicate that there are functional benefits to planting phylogenetically diverse seed mixes, even though many planted species may not emerge or persist (Arroyo-Rodríguez *et al.* 2012; Srivastava *et al.* 2012).

That seed mix diversity did not predict total plant community diversity (similarly to other studies, e.g., Barber *et al.* 2016), indicates that colonization by volunteer species can increase or decrease measures of phylogenetic diversity depending on the phylogenetic position of volunteers relative to the planted community. In addition to non-native species, we identified native volunteers in restored communities. These species were rare in seed mixes but found at numerous restorations, e.g., *Eupatorium altissimum* and *Asclepias syriaca*. It is unknown whether volunteer species colonized restored sites through

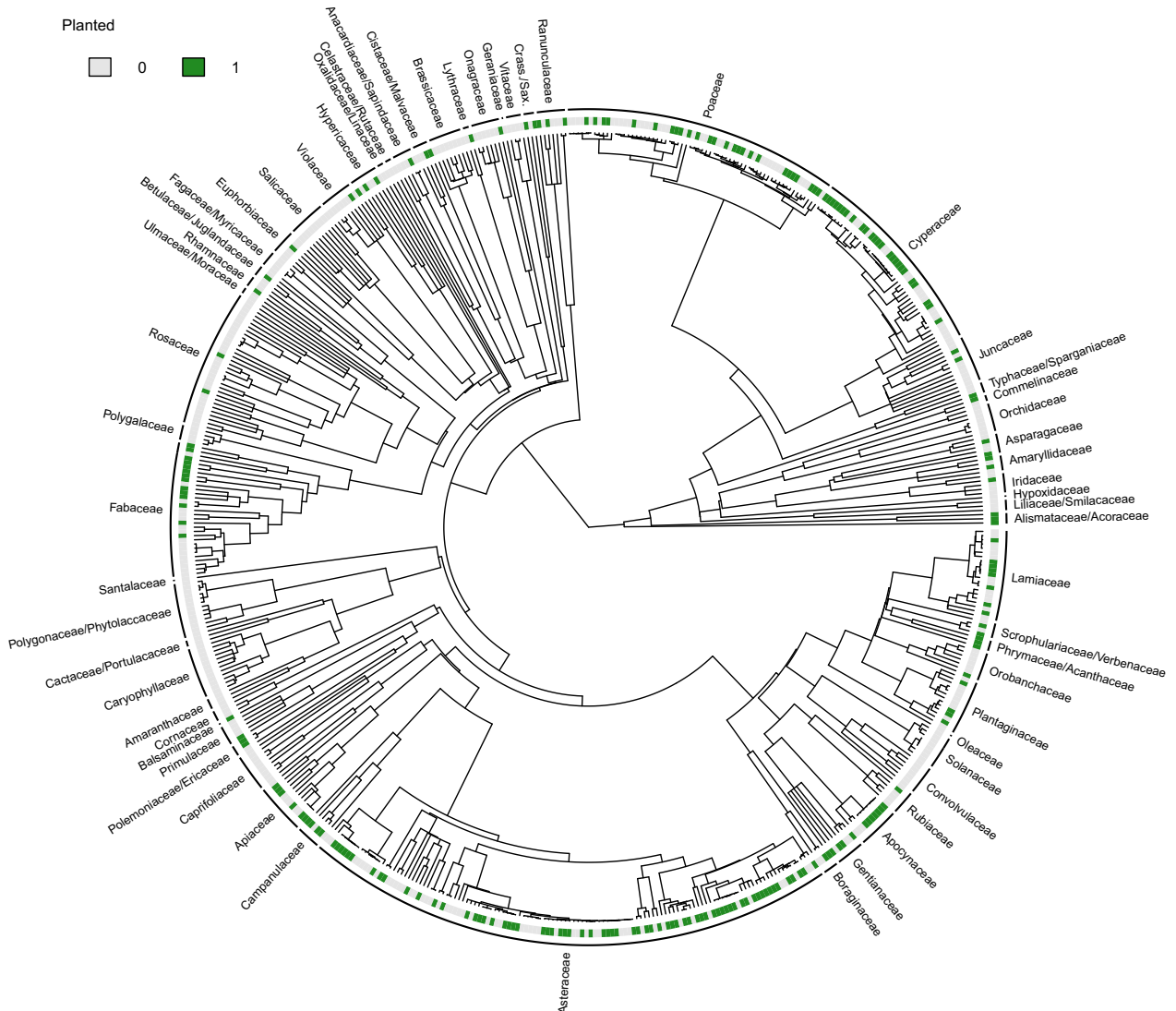


Fig. 4. Planted (green) and unplanted (grey) species are indicated on the phylogeny containing all species found at remnant and restored sites and seed mixes ($n = 589$). Planted species are phylogenetically clustered relative to a random null model ($D = 0.65$, $P < 0.0001$). Crass./Sax. = Crassulaceae/Saxifragaceae.

natural dispersal, or were deliberately introduced after initial seed mix determination. Additional study of establishment dynamics at restorations would help to clarify these patterns.

Colonization by volunteer species may explain why plant communities at restored sites tended to converge regardless of the seed mix originally used. Taxonomic and MNTD-based community results showed similar patterns, with little overlap between seed mixes and sites. In contrast, MPD-based analyses showed greater overlap between seed mixes and sites. We interpret these differences to mean that seed mixes and their resultant restored prairies are composed primarily of species from major families of the prairie (i.e., Asteraceae, Poaceae, Fabaceae and Lamiaceae), but that there is turnover in the particular species representing these families. This is because MPD measures reflect patterns of branching deep within a phylogeny (e.g., within families), while MNTD measures

reflect more recent branching, towards the tips of the phylogeny (e.g., within genera) (Tucker *et al.* 2016). We found that seed mixes and sites showed considerable overlap with respect to MPD, but diverged in terms of MNTD and SR, which is neutral with respect to evolutionary relatedness.

Our finding that clades found in remnant prairies were absent from seed mixes highlights opportunities to enrich the palette of plant materials used for restoration. There are multiple explanations for why species from these clades may have been missing, including lack of commercial availability, high cost or inadequate source populations (Peppin *et al.* 2010; Rowe 2010). Notable examples of missing families include Lilaceae and Orchidaceae, both of which contain species of regional conservation concern in remnant prairies. These species may have more precise requirements for germination and growth, making them difficult to source as seed and establish in restorations.

Seed and/or habitat modifications may be needed to facilitate their use in restoration (e.g., Bowles *et al.* 2005; Ault & Siqueira 2008). Hemiparasites can be important for ecosystem function and restoration of biodiversity (Pywell *et al.* 2004; Decler, Bonte & van Diggelen 2013); several species from missing clades are hemiparasites, including *C. umbellata* (Santalaceae) and multiple native *Cuscuta* (Convolvulaceae) species. Focusing on representatives of missing clades could be a means to increase phylogenetic diversity and associated function, while also supporting species-specific conservation goals.

IMPLICATIONS FOR MANAGEMENT AND RESTORATION

Incorporating phylogenetic diversity considerations into ecological restoration could advance management in at least two significant ways. First, increasing phylogenetic diversity of restorations may increase performance of key ecosystem functions (Cavender-Bares & Cavender 2011; Hipp *et al.* 2015). Second, phylogenetic ecology enriches understanding of differences between communities, offering new opportunities to monitor and evaluate performance of restored sites.

Our finding that restorations have lower phylogenetic diversity than remnants at multiple spatial scales and by multiple metrics indicates that understanding the factors that drive community phylogenetic patterns of restorations is important for building restorations that are predictably more similar to remnants. We found that seed mix diversity predicts established diversity for a range of diversity metrics. More detailed study of seeding techniques and outcomes would refine understanding of relationships between seed mixes and restored plant communities. For example, restorations established through multiple, rather than single, seeding events, are taxonomically more similar to high-quality reference prairies (W. Sluis, M. Bowles, and M. Jones, unpublished data). Similar patterns may emerge with respect to restoration of phylogenetic diversity.

Identifying underrepresented or missing clades in restoration inputs through phylogenetic comparison with reference sites can inform species selection for restoration. Increasing equivalence of restorations' phylogenetic diversity to that of remnants requires redesigning seed mixes to include species from clades not typically incorporated into restoration, and could include emphasis on planting species of conservation concern.

Furthermore, phylogenetic diversity can be a useful component of ongoing monitoring for restoration (Montoya, Rogers & Memmott 2012; Barak *et al.* 2016). Species from missing clades could be added in later stages of restoration. There are potential constraints to including these species in restoration, including low availability and/or low establishment. However, in the short term, planting plugs, rather than seeds, of desired species from underrepresented clades may help increase phylogenetic diversity, and ecosystem function, at restored sites

(Middleton, Bever & Schultz 2010; Gallagher & Wagenius 2016).

Authors' contributions

R.B., E.W., A.H., M.B. and D.L. conceived the ideas and designed methodology; R.B., E.W., G.C. and R.S. collected the data; R.B. analysed the data; R.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Plant community data, seed mix data, soil data and the phylogenetic tree are archived in Dryad, <https://doi.org/10.5061/dryad.90124> (Barak *et al.* 2017). Remnant data are already published and accessible at: <https://doi.org/10.5061/dryad.763v6> (Larkin *et al.* 2015b).

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Supporting Information

Details of electronic Supporting Information are provided below.

Table S1. Site information for restored prairies.

Table S2. Species found at remnant and restored sites, and seed mixes.

Table S3. Compositional differences between remnant and restored prairies.

Table S4. Site characteristics and biodiversity.

Table S5. Soil rda results.

Appendix S1. Additional diversity metrics.