## TOWARD PREDICTION IN THE RESTORATION OF BIODIVERSITY

# Dispersal and establishment limitation slows plant community recovery in post-agricultural longleaf pine savannas

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### Summary

**1.** Abandoned agricultural lands often have distinct plant communities from areas with no history of agriculture because plant species fail to recolonize. This may be due to dispersal limitation from a lack of seeds, or establishment limitation because of unsuitable environmental conditions. However, few experiments have directly tested how restoration activities may overcome these limitations.

**2.** We studied longleaf pine savannas in South Carolina abandoned from agriculture > 60 years ago that were immediately adjacent to remnant habitats (areas with no history of agriculture). Using 27 sites, we conducted a factorial experiment that sowed seeds of 12 species indicative of remnant communities and conducted restoration thinning of overstorey trees in half of 126, 1-ha patches to mimic canopy density of natural savannas. We also established vegetation transects to examine if restoration promotes spread of remnant species into post-agricultural areas.

**3.** We found strong evidence for dispersal limitation in post-agricultural areas as over 99% of the occurrences of our focal species were in seed addition plots. Seed additions increased total species richness by 27%.

**4.** Restoration thinning increased establishment in seed addition plots (measured as richness of sown species) by 126% and increased total richness by 88%. Restoration thinning also increased seed production in remnant habitats by an average of 6506% across our focal species. However, after 4 years, restoration thinning did not facilitate the natural spread of remnant species into adjacent post-agricultural sites.

**5.** *Synthesis and applications.* We show that both dispersal and establishment limitation are key factors causing some plant species to be absent from post-agricultural sites. Dense canopy conditions limit seed production in remnant habitats and reduce establishment in post agricultural areas. Restoration thinning helps overcome these limitations and should facilitate the natural spread of species from remnant habitats but natural recovery may still be slow. Our results suggest that accelerating the recovery of post-agricultural habitats will require active restoration that reduces dispersal limitation (seed additions) and reinstates appropriate ecological conditions.

**Key-words:** biodiversity conservation, community assembly, community ecology, dispersal, establishment, land-use legacies, longleaf pine savanna, post-agriculture, restoration ecology, seed addition

## Introduction

Legacies of human land use on natural systems are widespread, long-lasting, but still poorly understood (Foster

*et al.* 2003; Flinn & Vellend 2005; Cramer, Hobbs & Standish 2008). Intensive land-use activities, such as tillage agriculture and grazing, cover over 30% of Earth's land area, and have well-recognized dramatic impacts on species diversity and composition, as well as aspects of ecosystem functioning (Vitousek 1997; Foley *et al.* 2005; Newbold *et al.* 2015). A more recent realization is that

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these habitat modifications have lasting impacts on recovering ecosystems, even long after intensive land use ceases (Foster *et al.* 2003; Flinn & Vellend 2005; Hermy & Verheyen 2007; Perring *et al.* 2016). For example, many plant species fail to recolonize abandoned agricultural lands, even hundreds or thousands of years following agricultural abandonment (Dupouey *et al.* 2002; Vellend *et al.* 2006; Brudvig & Damschen 2011; Brudvig *et al.* 2013; Freschet *et al.* 2014). In spite of the ubiquity of this pattern, the factors preventing post-agricultural recovery remain poorly understood due to a lack of experiments testing putative mechanisms. Understanding the constraints to post-agricultural recovery will, in turn, be critical for guiding restoration to promote native biodiversity in post-agricultural landscapes (Cramer, Hobbs & Standish 2008; Suding 2011).

Limited post-agricultural plant community recovery may be due to two mechanisms: dispersal limitation and establishment limitation (Table 1) (Flinn & Vellend 2005; Hermy & Verheyen 2007; Standish *et al.* 2007; Öster *et al.* 2009). Dispersal limitation – lack of seed arrival to a location – may result from the inability of a species to disperse into a post-agricultural area or a lack of seed sources (reproductive individuals) in the surrounding area. Establishment limitation results when individuals arrive, but fail to survive or reproduce due to inadequate environmental conditions (Crawley 2000; Orrock *et al.* 2006). Seed addition experiments can test the relative importance of both mechanisms. Comparing between seed addition plots and non-seed addition plots informs aspects of seed limitation while comparing among seed addition plots in different habitats informs how those environmental conditions influence establishment (Table 1). These approaches have been widely employed in ecological research (Turnbull, Crawley & Rees 2000; Clark *et al.* 2007), yet land-use legacy studies have often attempted to infer dispersal and establishment limitation observationally (Hermy & Verheyen 2007; De Frenne *et al.* 2010; Brunet *et al.* 2012). When conducted, seed addition and transplant experiments demonstrate that both dispersal and establishment limitation are important in preventing community recovery in post-agricultural landscapes (Graae, Hansen & Sunde 2004; Standish *et al.* 2007; Cousins &

Lindborg 2008; Baeten *et al.* 2009; Öster *et al.* 2009; Jackson, Pearson & Turner 2013; Hahn & Orrock 2016). But the mechanisms causing this limitation and the interactive effects of dispersal and establishment limitation remain poorly resolved.

Understanding the roles of dispersal and establishment limitation will help to guide restoration of post-agricultural ecosystems and, in turn, habitat restoration may help to mitigate land-use legacies by either directly or indirectly overcoming establishment and/or dispersal limitation. The persistence of land-use legacies suggests that in many cases, passively allowing post-agricultural ecosystems to recover will not be effective (Foster *et al.* 2003; Flinn & Vellend 2005; Hermy & Verheyen 2007; Cramer, Hobbs & Standish 2008; Brudvig *et al.* 2014). Active restoration approaches may take many forms, however, and little is known about how different approaches serve to encourage the dispersal and establishment of sensitive species into post-agricultural, or otherwise degraded, habitats (Fig. 1). Adding species, for example, through seed additions, may directly reduce dispersal limitation, but inappropriate environmental conditions may prevent reestablishment into post-agricultural areas, rendering seeding alone ineffective. As such, restoring appropriate habitat conditions in post-agricultural areas may be critical for alleviating establishment limitation (Fig. 1c). Moreover, seed additions represent an intensive and expensive restoration activity. Relict populations persisting in remnant habitats (areas with no history of agriculture) may serve as natural seed sources and restoration of remnant areas may alleviate dispersal limitation by increasing seed export to post-agricultural areas (Fig. 1b). In some cases it may be necessary to restore both the remnant and post-agricultural areas to reduce both dispersal and establishment limitations (Fig. 1d). Identifying the combination of techniques that best addresses both dispersal and establishment limitations will be necessary to restore biodiversity to post-agricultural landscapes.

Here, we experimentally evaluate the relative roles of dispersal limitation and establishment limitation for maintaining post-agricultural land-use legacies and the prospects for restoration activities to alleviate these legacies, within longleaf pine savannas. Longleaf pine savannas are

**Table 1.** Hypotheses for why species fail to spread from remnant habitats to nearby post agricultural areas

| Hypotheses | Experiment to test hypothesis | Expected outcome if hypothesis is supported |
|---|---|---|
| *Dispersal limitation* | | |
| Lack of seed production in remnant habitats | Restoration thinning in remnant habitats | Plants in restored remnant habitats will have increased seed production compared to unrestored |
| Seeds not arriving in post-ag. habitats | Seed additions in post-ag. habitats | Seed addition plots will have more recruits than control plots |
| *Establishment limitation* | | |
| Unrestored habitat conditions prevent establishment | Couple habitat restoration with seed additions in post-ag. habitats | More sown recruits will establish in restored post-ag. habitats compared to unrestored post-ag. habitats |
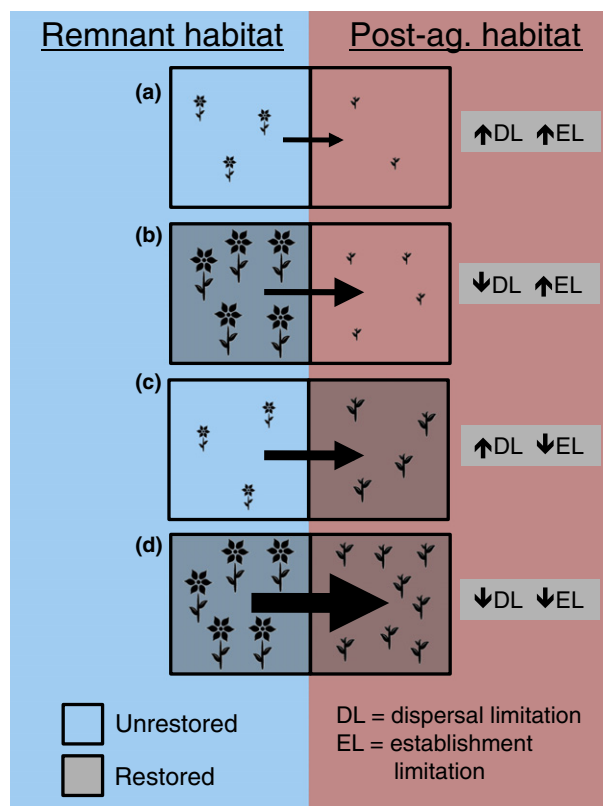
**Fig. 1.** A conceptual model of how restoration is expected to facilitate the recovery of post-agricultural habitats by promoting the spread of species indicative of nearby remnant habitats. The expected amount of recolonization by remnant species is depicted by the size of arrows and is limited by either the ability of species to get to an area (dispersal limitation, represented by the size of flowering plants remnant habitats) or the ability to survive in that habitat (establishment limitation, represented by the size of seedlings in post-agricultural habitats) both of which may be pronounced without restoration (a). If remnant habitats are degraded in some way, then restoration in those habitats could increase seed production and help decrease dispersal limitation (b). Active restoration in post-agricultural areas should make those habitats more hospitable to target species thus decreasing establishment limitation (c). The most recolonization of remnant species is expected when both habitats are restored because this would decrease both dispersal and establishment limitation (d). [Colour figure can be viewed at wileyonlinelibrary.com]

a component of the Southeastern United States global biodiversity hotspot (Noss *et al.* 2014) which are seriously threatened by past and current land-use activities. Less than 3% of the historical ecosystem range remains intact due to conversion to agriculture and other human land uses, as well as alterations to the fire regime. Understorey fire as frequent as every 1–3 years is necessary to maintain diversity and open-canopy structure (Jose, Jokela & Miller 2006). Although few areas have escaped some form of intensive land use, much of the area formerly under agricultural cultivation has been abandoned or converted to native pine plantations, presenting opportunities for habitat restoration (Brudvig & Damschen 2011). Previous work in this system shows the importance of fire, soils,

tree canopy cover and landscape structure, among other factors, in shaping plant communities and plant establishment in both remnant and post-agricultural habitats (Kirkman *et al.* 2001, 2004; Mattingly & Orrock 2013; Brudvig *et al.* 2014; Veldman *et al.* 2014). However, despite this progress, little is known about how multiple restoration techniques (e.g. seed additions paired with habitat modifications) influence establishment and dispersal limitation and how to best facilitate the spread of plant species from remaining remnant habitats into post-agricultural areas.

To address these gaps we worked in post-agricultural longleaf pine savannas adjacent to remnant sites with no history of agriculture and conducted large-scale habitat restorations (tree removal which we call 'restoration thinning'), seed additions, and vegetation surveys. Previous work in this system found that post-agricultural and remnant habitats had distinct understorey plant community compositions and that restoration thinning increased diversity but did not make these communities more similar (Turley & Brudvig 2016). In this study, we build upon those results by focusing on a subset of plant species that are responsible for this trend, specifically, species that tend to be restricted to remnant habitats, that is, remnant indicator species (Brudvig *et al.* 2013). We asked four questions: (i) Is there evidence for dispersal limitation in post-agricultural sites? We tested this using seed addition experiments in post-agricultural patches. (ii) Does restoration thinning increase reproductive output? We tested this by evaluating the effects of tree removal on floral production of understorey plants, which we use as a proxy for reproductive output. (iii) Does restoration thinning decrease establishment limitation? We tested this by comparing outcomes of seed additions in both restored and unrestored post-agricultural areas. And finally, (iv) Does restoration thinning, either in remnant or post-agricultural patches, promote spatial spread into post-agricultural areas? We tested this by surveying plant communities in adjacent remnant and post-agricultural patches, which were either restored or unrestored.

## Materials and methods

### STUDY SYSTEM

We conducted our research in longleaf pine savannas at the Savannah River Site (SRS), a National Environmental Research Park in South Carolina (33·20°N, 81·40°W). Our 27 sites were distributed across 802 km$^2$ and each had a patch of remnant longleaf pine savanna adjacent to post-agricultural pine plantation. The post-agricultural areas were historically in tillage agriculture, which was abandoned at all sites when SRS was created in 1951. Abandoned agricultural fields were subsequently managed as longleaf and loblolly pine plantations. Remnant sites were forested in 1951, based on aerial photography, have no known history of agriculture, and do not differ from post-agricultural sites in their soil types (Brudvig *et al.* 2013). Prior to this study, all sites showed evidence of fire suppression,

including canopy closure and leaf litter build up. At each site, we established a total of 4–10 (depending on the size of the remnant and post-agricultural areas) 1-ha (100 m × 100 m) research patches, with patches paired on either side of the land-use boundary (Fig. 2). This resulted in a total of 126 patches. For further details about establishment criteria see Brudvig *et al.* (2013). Our previous research in these patches showed strong effects of land-use history on plant communities and environmental conditions. We found distinct changes in plant communities only 10 m away from the land-use boundary (Grman *et al.* 2015), and of the over 300 plant species found in our surveys over 50 were found predominantly in remnant sites and over 40 were found predominantly in post-agricultural sites (Brudvig *et al.* 2013). We also found that remnant sites had higher canopy cover, duff depth, soil water holding capacity, and soil organic matter, while post-agricultural sites had higher soil phosphorous and leaf litter depth (Brudvig *et al.* 2013) suggesting there are lasting abiotic differences between these habitats that could shape understorey plant establishment and performance.

### RESTORATION THINNING

To test how restoration practices influence recolonization of post-agricultural sites, we conducted restoration thinning to reinstate open-canopy conditions indicative of longleaf pine savannas in our region (Jose, Jokela & Miller 2006). Thinning occurred within half the experimental patches in 2011 and was implemented in a randomized 2 × 2 split-plot design, resulting in each site supporting four treatment combinations of overstory tree thinning and agricultural history (Fig. 2). To test the effects of restoring adjacent habitats on recolonization we varied the spatial arrangement of thinning treatments across sites. Thinned and unthinned post-agricultural patches were either adjacent to thinned or unthinned remnant patches, as in Fig. 1. The overstory was mechanically thinned

to 10 longleaf pine trees per hectare, with cut trees removed from each site. Prior to thinning, tree stem densities averaged ~650 per ha and tree basal areas averaged ~20 m$^2$ ha$^{-1}$, with neither measure differing between remnant habitats and post-agricultural plots (Brudvig *et al.* 2013).

### REPRODUCTIVE OUTPUT OF FOCAL SPECIES

We conducted field surveys to understand how restoration thinning influenced reproductive output of understorey plant species. We chose 11 focal species that, in 2010, were found primarily in remnant habitats (Brudvig *et al.* 2013). These included four grasses in the Poaceae (*Anthaenantia villosa, Sorghastrum nutans, Sorghastrum secundum,* and *Sporobolus junceus*), six forbs in the Asteraceae (*Carphephorus bellidifolius, Coreopsis major, Eupatorium album, Silphium compositum, Solidago erecta* and *Vernonia angustifolia*) and one forb in the Polygonaceae (*Eriogonum tomentosum*). We focused primarily on Poaceae and Asteraceae because these are the two most species rich and among the most dominant families in this study system (Blake & Kilgo 2005). All focal species are perennial and are primarily passively dispersed, by wind and/or gravity. In fall 2013, we established 20 m × 60 m transects, parallel to our vegetation transects within thinned and unthinned remnant patches at 14 of 27 sites. Sites were selected based on the presence and abundance of these focal species. To quantify reproductive output, we counted the number of reproductive structures (flowers or inflorescences) within the transects for each species. In 10% of cases where individual species were very abundant we surveyed a smaller transect (10 m × 60 m or 10 m × 20 m); all measures were converted to number of flowering structures per 100 m$^2$. To test whether the number of floral structures is a good proxy for seeds produced across unthinned and thinned patches, we also counted the number of seeds within each floral structure (three per plant) from a subset of plants (three per transect) from four of our focal species. We multiplied the number of floral structures in each patch by the average number of seeds per structure in that patch to get an estimate of total number of seeds per 100 m$^2$ within each patch.

### SEED ADDITIONS

We collected seeds in fall 2011 from the 11 focal species mentioned above from at least three natural populations per species at SRS. We also obtained seeds of an addition species, *Aristida beyrichiana,* which were mechanically collected from a single site in fall of 2012. This site was originally planted and subsequently maintained by the U.S. Forest Service as a seed source for this species. For each species, we combined and mixed seeds from all populations. Seeds were subsequently agitated against wire mesh to remove them from glumes, floral structures, and chaff where necessary. We then created one weighed seed packet for each species, to correspond with each seed addition plot in the field. We used this approach to standardize the number of seeds that were sown into a plot for each species, maximizing our ability to compare the effect of restoration thinning and seed addition treatments within a species, consistent with the primary goals of our study (Table 1). Based on seed counts from a subset of 10 of these packets, the average number of seeds per packet for each species was 300 (range = 106–716, SD = 173).

To experimentally assess the roles of dispersal limitation and establishment limitation for generating the pattern of constrained
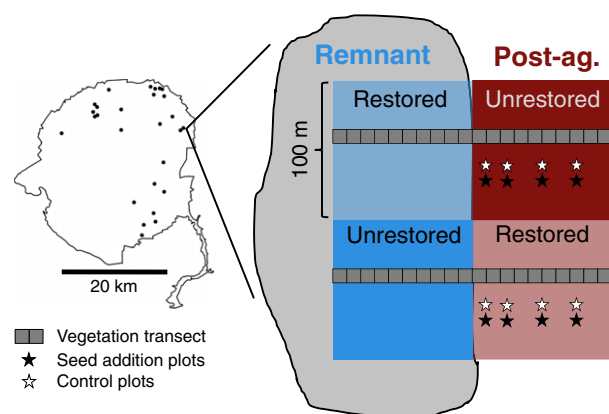


**Fig. 2.** Diagram of our study location and an example of one of our research sites. All of our study sites are at the Savannah River Site in South Carolina, USA, depicted on the left. Each dot represents 1 of our 27 research sites, which each have a remnant area, which has never been in agriculture, and an adjacent post-agricultural area, which were all abandoned in the 1950s. At each site we established 1-ha (100 × 100 m) patches that have a transect of 10 successive 10 × 10 m vegetation plots. We also established seed-addition and paired control plots, in each post-agricultural plot at 10, 25, 50 and 75 m from the land-use boundary. [Colour figure can be viewed at wileyonlinelibrary.com]

spread of remnant populations into post-agricultural areas, we added seeds of remnant species to plots within all 1-ha post-agricultural patches in February 2013. Using data from a nearby National Climate Data Center station we found that monthly precipitation in 2013 was on average 2·5 cm higher than the 1981–2010 average but this trend varied widely from month to month (SD = 8·4); total precipitation was 27% higher. We established 1 × 1 m seed addition and adjacent control plots 10, 25, 50 and 75 m from the land-use boundary (Fig. 2) for a total of 252 seed addition plots. Seeds of all 12 species were sprinkled onto the ground from prepared packets, resulting in approximately 3600 seeds added per 1 $m^2$ in each seed addition plot. In 2015, we surveyed the presence of all vascular plant species with tissue rooted in or overhanging less than 1·3 m in height in 0·5 $m^2$ (0·71 × 0·71 m) subplots centred within the seed addition and control plots. We also counted the number of individuals of each of our focal species rooted inside the 0·5 $m^2$ subplots.

### VEGETATION SURVEYS

To understand if remnant species are spreading into the post-agricultural sites, we established 100-m-long vegetation sampling transects perpendicular to land-use boundaries (Fig. 2). Each transect contained ten contiguous 10 × 10 m subplots. In 2010, we recorded the presence of all ground-layer vascular plant species rooted within or overhanging each 10 × 10 m subplot. These 2010 data served as a baseline and the same transects were resurveyed in 2015 to explore how plant communities changed in response to the restoration thinning manipulation explained above.

### DATA ANALYSES

To evaluate patterns of spatial spread we focus on species that had a significant association with remnant patches. We determined which species are indicators of remnant patches using the baseline 2010 vegetation data and a point-biserial correlation coefficient indicator analyses using the 'strassoc' function with an alpha value of 0·05 (de Cáceres & Legendre 2009), which resulted in 60 remnant indicator species (Appendix S1, Table S1, Supporting Information). We tested whether the richness of these species in 2015 in our 1000-$m^2$ vegetation transects was affected by land-use history, restoration thinning, whether the adjacent remnant patch was restored and distance from land-use boundary.

To test how our treatments influenced richness of remnant indicator species we fit a nested ANOVA using the 'lme' function using the model formula syntax: remnant indicator richness ~ (land use + restoration + adjacent restoration + distance)^3, random = ~1|site/land use/restoration/distance. This model tests the main effects and all three-way interactions for the fixed effects, and has nesting in the random effects ('distance' within 'restoration' within 'land use' within 'site') to account for pseudoreplication inherent in split-plot experimental designs. In this model, 'restoration', 'adjacent restoration' and 'land use' are two-level categorical predictors, distance is a continuous variable, and 'site' is a 27-level categorical predictor with one level for each of our independent replicates. We simplified the model first by removing all three-way interactions that were not significant ($P > 0·05$) followed by removing all non-significant two-way interactions. To get a measure of variance explained for each interaction factor we used the 'r.squaredGLMM' function (Nakagawa & Schielzeth

2012) and used the difference in model marginal $R^2$ between the full model and the models with each factor removed; for main effects we used the difference in model $R^2$ between the model with all fixed effects and models with each main effect removed.

To test the roles of seed additions and restoration thinning for the richness of our focal species and total species richness in post-agricultural patches, we fit models with the following syntax: richness ~ seed addition × restoration, random = ~1|site/restoration/seed addition). In these models 'seed addition' is a two-level categorical factor and the other factors are the same as in the model described above. For these models we report results for interaction terms from full models and results for the main effects from models with non-significant ($P > 0·05$) interaction terms removed. For all models we tested significance of each factor using the 'anova.lme' function with marginal (type III) sums of squares. All models fit assumptions, as residuals were normally distributed and there was limited heteroscedasticity in the relationship between fitted values and residuals.

For each focal species we tested how restoration thinning affected reproductive output by calculating the per cent effect of the restoration treatment on number of floral structures or seed number at each site as: (mean value in restored−mean value in unrestored)/mean for that species across all unrestored transects × 100. We then calculated an average effect size and 95% confidence interval (standard error × 1·96) using the effect size from each site as replicates. All analyses were done using R version 3.2.2. All the original data used for analyses are available online (Turley *et al.* 2017).

## Results

### IS THERE EVIDENCE FOR DISPERSAL LIMITATION IN POST-AGRICULTURAL SITES?

We found strong evidence for dispersal limitation as seed addition plots had higher sown, and total, richness than control plots. Over 99% of the occurrences of our focal species (1193 out of 1197) were in seed addition plots rather than control plots (Fig. 3a). Adding seeds to unrestored plots of 12 species resulted in an average of 1·1 focal species per plot ($F_{1,52} = 20·7$, $P < 0·001$) and, across plot types, seed additions increased total species richness by 27% ($F_{1,53} = 29·6$, $P < 0·001$; Fig. 3b).

### DOES RESTORATION THINNING INCREASE REPRODUCTIVE OUTPUT?

Restoration thinning greatly increased the number of floral structures in longleaf pine savanna understorey plants. Across 11 species the number of floral structures per 100 $m^2$ increased by an average of 6506% in restored patches compared to unrestored patches and this was a significant increase in nine of the species (i.e. 95% confidence interval did not overlap with zero; Fig. 4). For the subset of species where we estimated the total number of seeds per 100 $m^2$ we found that thinning increased average seed production by 2881% (*C. bellidifolius* = 778 ± 451, *S. erecta* = 3812 ± 3458, *S. secundum* = 676 ± 554, *S. junceus* = 6256 ± 6459; mean ± 95% CI).

**Fig. 3.** Interactive effect of seed additions and restoration thinning on species richness of sown species (a) and total species richness (b) in 0·5 m² plots. (a) Sown species were almost entirely absent in control plots and thinning increased richness of species in seed addition plots by 126%. (b) Total species richness was 27% higher in sown plots and 88% higher in thinned plots. Both figures show the results of nested ANOVAs testing effects of thinning, seed addition and their interaction on richness. [Colour figure can be viewed at wileyonlinelibrary.com]
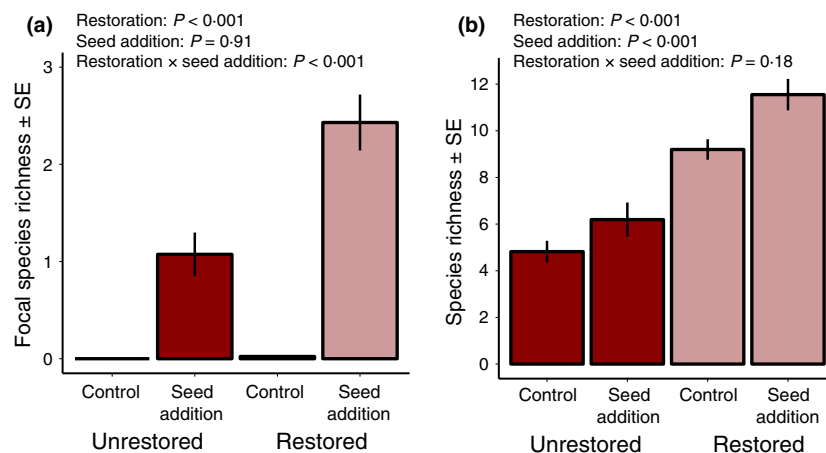
### DOES RESTORATION THINNING DECREASE ESTABLISHMENT LIMITATION?

We found that restoration thinning increased establishment of our focal species when seeds were added and increased overall richness. Seed addition plots in restored patches had an average 2·4 focal species which is 126% larger than in seed additions plots in unrestored patches; however, restoration thinning did not increase focal richness in our control plots (restoration × seed addition, $F_{1,52} = 15·3$, $P < 0·001$, Fig. 3a). Restoration thinning also increased total species richness by 88% ($F_{1,26} = 74·5$, $P < 0·001$; Fig. 3b).

### DOES RESTORATION THINNING PROMOTE SPATIAL SPREAD INTO POST-AGRICULTURAL AREAS?

We found no evidence that restoration thinning promotes spread of remnant indicator species into adjacent post-agricultural habitats. In unrestored patches we found a pronounced spatial signature in plant species' occurrences when crossing the land-use boundary between remnant sites and post-agricultural sites. Remnant indicator species, as determined from 2010 data, were not spreading from remnant patches into adjacent post-agricultural patches by 2015. Remnant indicator richness in post-agricultural plots 10 m away from remnant habitats decreased by 62% compared to average in remnant plots (Fig. 5). These patterns were supported by a significant distance × land use interaction in our model, which explained 2% of the variation in remnant indicator richness (Table 2). We found that restoring the adjacent remnant patch had no effect on richness of remnant indicators in post-agricultural patches (Table 2) and we saw no difference in the richness of remnant indicator species in restored and unrestored post-agricultural patches (Table 2, see dashed red line in Fig. 5). Our final model did include several significant interaction terms (Table 2) containing the restoration thinning factor but these were the result of small differences in means among treatments (<1 species) and they all explained little variation (<0·5%).

## Discussion

Human activities have long-lasting impacts on biodiversity, especially intensive habitat modification like agriculture (Foster *et al.* 2003; Hermy & Verheyen 2007;

**Fig. 4.** Restoration thinning increased the number of floral structures of 11 understorey plant species by an average of 6506%. The effect of thinning on floral production was determined by counting floral structures in a standardized area in both thinned and control plots. *X*-axis show species codes (first three letters of genus and species) see methods section for full species names.
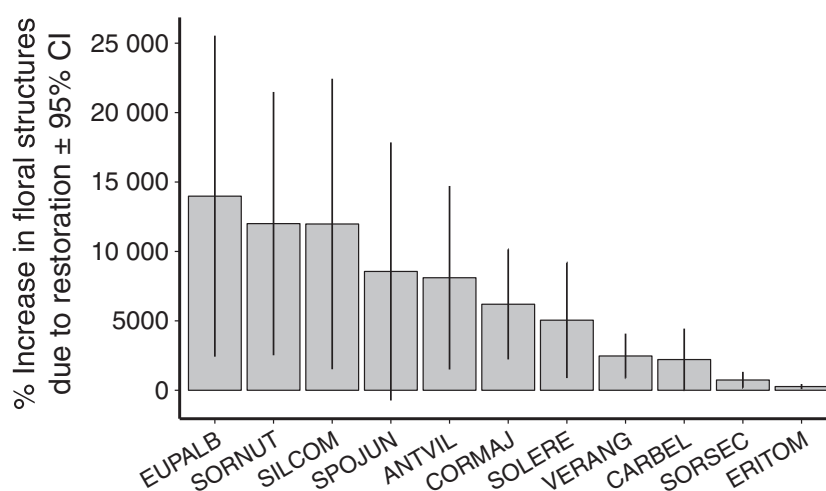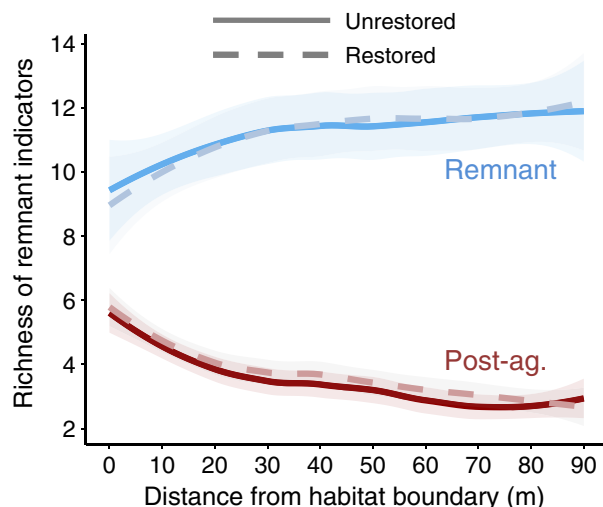
**Fig. 5.** Spatial patterns in the richness of 60 remnant indicator species (species indicative of remnant sites in 2010) in 10 × 10 m plots. Remnant indicator richness increased in post-agricultural habitats closer to the land-use boundary, but still remained much lower than in remnant habitats even right at the land-use boundary. Restoration thinning of canopy trees had no impact on richness of remnant indicator species in either habitat. Shaded areas are 95% confidence intervals [Colour figure can be viewed at wile yonlinelibrary.com]

Brudvig *et al.* 2014). In our system, agricultural land-use legacies shape plant communities, but we found that active restoration techniques can help overcome these effects. The highest number of remnant indicator species established in post-agricultural areas when experimental seed additions were combined with restoration thinning, but establishment of sown species was still low suggesting that various factors are still limiting recruitment. We also explored how the immediate proximity of post-agricultural areas to remnant areas might facilitate community recovery and whether active restoration can expedite that process. We found that restoration thinning in both remnant and post-agricultural habitats increased reproductive output and establishment when seeds were added; however, after 4 years, we have yet to see impacts of restoration thinning on spatial spread of remnant species, likely because not enough time has passed for natural recruitment to occur in post-agricultural areas.

### DISPERSAL AND ESTABLISHMENT LIMITATION IN POST-AGRICULTURAL HABITATS

Two lines of evidence suggest that dispersal limitation is preventing the spread of remnant species into post-agricultural areas in this system. First, we found strong seed limitation of remnant indicator species in post-agricultural plots, even when only 10 m from the land-use boundary (Fig. 3). This mirrors results seen in similar studies in longleaf pine savannas, grasslands and deciduous forests (Ehrlén & Eriksson 2000; Graae, Hansen & Sunde 2004; Kirkman *et al.* 2004; Verheyen & Hermy 2004; Standish

**Table 2.** Results of a nested ANOVA testing how richness of remnant indicator species in 10 × 10 m plots is affected by 'Land use' (remnant and post-agricultural habitats) and 'Restoration' (patches with and without tree thinning). The model also included 'Distance' (distance of a plot from the land-use boundary) and 'Adj. restoration' (whether the adjacent patch on the other side of the land-use boundary was a restored or unrestored patch). Land use had by far the largest impact on remnant indicator richness. The Distance × Land use interaction explained the second-largest amount of variation (see Fig. 5)

| | nd.f. | dd.f. | f | P | $R^2$ |
|---|---|---|---|---|---|
| Restoration | 1 | 52 | 0·64 | 0·427 | <0·001 |
| Land use | 1 | 26 | 52·10 | <0·001 | 0·513 |
| Distance | 1 | 970 | 61·09 | <0·001 | <0·001 |
| Adj. restoration | 1 | 136 | 0·11 | 0·746 | <0·001 |
| Land use × Adj. restoration | 1 | 136 | 7·59 | 0·007 | <0·001 |
| Land use × Distance | 1 | 970 | 114·39 | <0·001 | 0·020 |
| Restoration × Land use | 1 | 52 | 11·97 | 0·001 | <0·001 |
| Restoration × Land use × Adj. restoration | 2 | 136 | 15·48 | <0·001 | 0·004 |

*et al.* 2007; Kardol *et al.* 2008; Öster *et al.* 2009; Hahn & Orrock 2016). Second, we found low seed production in closed-canopy remnant patches compared to restored open-canopy patches (Fig. 4), which could be a cause of seed limitation in post-agricultural patches. Intact longleaf pine savannas typically have sparse tree cover but fire suppression leads to encroachment of hardwood trees and closed canopy conditions (Jose, Jokela & Miller 2006). Given that these remnant habitats have a long history of fire suppression and closed-canopy conditions, long before the abandonment of agriculture, there has likely been low seed production and thus limited opportunities for seeds to disperse to adjacent post-agricultural areas (Kirkman *et al.* 2004). This could explain why many species had failed to spread even a few meters into post-agricultural sites after over 60 years (Fig. 5). Other observational studies in a variety of systems have found similar spatial patterns (Hermy & Verheyen 2007; Cousins & Lindborg 2008; Öster *et al.* 2009; De Frenne *et al.* 2010; Brunet *et al.* 2012).

In addition to seed limitation, we found that remnant species may have failed to spread because closed-canopy post-agricultural habitats were unsuitable for establishment following seed arrival. Restoration thinning increased the richness of sown species in seed addition plots by 88% (Fig. 3) and increased the total number of individuals of those species in our plots from 321 to 876. Previous work in this system also found increased establishment and growth following seed additions and transplants in restored patches, although these effects were stronger in remnant habitats than in post-agricultural areas (Hahn & Orrock 2016). Together these results suggest that above- and below-ground competition by trees limit establishment in post-agricultural habitats; but other factors such as competition from other species in the

understorey (Endels *et al.* 2004), natural enemies (Orrock, Witter & Reichman 2009; Hahn & Orrock 2016), increased soil phosphorous levels (Dupouey *et al.* 2002; Brudvig *et al.* 2013) and altered soil microbial communities (Jangid *et al.* 2011) could also be important. Temporal variation in environmental conditions may also be important in contributing to the patterns of establishment we observed (Fukami 2015). For example, in the year that we performed our seed addition experiment there was high levels of precipitation. Further work is needed to disentangle the relative importance of factors affecting establishment in post-agricultural habitats and over what time frames they are important. Moreover, given that our focal species are long-lived perennials, the effects of some of these other factors on continued recruitment and spread following establishment may only become apparent over a time period longer than the three growing seasons we studied them (Baeten, Hermy & Verheyen 2009).

### RESTORATION EFFECTS ON NATURAL RECOLONIZATION OF POST-AGRICULTURAL AREAS

The restoration of biodiversity on post-agricultural areas can be facilitated by nearby remnant habitats, or otherwise less degraded, habitats because they can serve as source populations (Kirkman *et al.* 2004; Cousins & Lindborg 2008; De Frenne *et al.* 2010). But when species from remnant habitats fail to naturally colonize post-agricultural areas, active restoration can help recover biodiversity in post-agricultural habitats by helping to overcome both dispersal and establishment limitation (Fig. 1) (Verheyen & Hermy 2004). In our system, we predicted that restoration thinning would increase seed production of remnant species and increase establishment when seeds were added to post-agricultural areas, and that these, separately or together, would increase the spread of remnant species into post-agricultural areas (Fig. 1b–d).

We found support for some of these predictions but not others. While restoration thinning did create conditions that should facilitate spread (increased seed production and increased establishment success when seeds were added) we did not see any effect of restoration thinning, whether in the post-agricultural patch or adjacent remnant patch, on the natural spread of remnant indicator species into post-agricultural areas. This could be because recruitment in this system is rare (Mulligan, Kirkman & Mitchell 2002). In our seed addition plots, for example, restoration thinning did increase establishment but we still only saw an average of 2·4 out of 12 species in each plot. Therefore, 4 years post-restoration may not be enough time to observe any effects of restoration thinning on spread. This may be especially true for species with low recruitment (Baeten *et al.* 2009) and limited dispersal capacity which may take several generations to move just a few meters (Kirkman *et al.* 2004; Baeten *et al.* 2015). There could also be other limiting factors, for example, the spread of tropical forest trees into pastures is shaped primarily by dispersal, but also competition, light levels, nutrients and herbivory (Holl 1999; Holl *et al.* 2000). In longleaf pine savannas, fire is critical for maintaining diversity and promoting many endemic species (Jose, Jokela & Miller 2006; Glitzenstein *et al.* 2012) but it is not known if fire will increase the rate of spread into post-agricultural areas. In this and other systems, experiments that manipulate a range of environmental conditions or restoration practices to see how they influence spatial spread out of remnant habitats, and over what timeframe, would be helpful in understanding how to best restore biodiversity in post-agricultural habitats.

### IMPLICATIONS FOR MANAGEMENT

Our research reinforces the idea that active restoration is often necessary to recover plant biodiversity in post-agricultural landscapes (Flinn & Vellend 2005; Hermy & Verheyen 2007). Evidence from many systems suggests that passive restoration approaches are often not effective because many native species fail to recolonize post-agricultural areas after decades or even centuries (Dupouey *et al.* 2002; Flinn & Vellend 2005; Hermy & Verheyen 2007; Cousins & Lindborg 2008). This pattern was evident in our system despite post-agricultural areas being immediately adjacent to remnant habitats which could serve as seed sources for those species (Brudvig *et al.* 2013; Grman *et al.* 2015). Our research suggests that seed additions or transplants (Aschenbach, Foster & Imm 2010) may be necessary to establish sensitive and long-lived longleaf pine understorey species outside of remnant habitats. These efforts will be facilitated by tree thinning, which reinstates the open-canopy conditions indicative of healthy longleaf pine savannas (Jose, Jokela & Miller 2006), and continued management with fire to prevent trees from returning (Glitzenstein *et al.* 2012). In the long term, we predict that thinning will help promote natural recolonization of post-agricultural habitats because it greatly increases seed production, promotes establishment and increases overall diversity (Brudvig & Damschen 2011; Veldman *et al.* 2014; Turley & Brudvig 2016), even though these factors are not promoting spread in the short term (Turley & Brudvig 2016). In other post-agricultural systems, combinations of seed additions and habitat restoration are expected to accelerate recovery as well (Verheyen & Hermy 2001; Flinn & Vellend 2005). By understanding the factors contributing to dispersal and establishment limitation, specific restoration techniques may be tailored to a variety of post-agricultural ecosystems (Holl *et al.* 2000; Öster *et al.* 2009).

## Authors' contributions

J.L.O. and L.A.B. conceived the study; J.A.L., J.L.O. and L.A.B. designed the experiments; J.A.L. collected the data; N.E.T. analysed the data and wrote the first draft of the paper. All authors revised the paper and gave final approval for publication.

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## Data accessibility

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.9g785 (Turley *et al.* 2017).

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## Supporting Information

Details of electronic Supporting Information are provided below.

**Appendix S1.** Explanation of columns in Table S1.

**Table S1.** List of plant remnant and post agricultural indicator values and frequencies.