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RESEARCH ARTICLE

Forest and Biodiversity 2: A tree diversity experiment to understand the consequences of multiple dimensions of diversity and composition for long-term ecosystem function and resilience

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Abstract

1. We introduce a new "ecosystem-scale" experiment at the Cedar Creek Ecosystem Science Reserve in central Minnesota, USA to test long-term ecosystem consequences of tree diversity and composition. The experiment—the largest of its kind in North America—was designed to provide guidance on forest restoration efforts that will advance carbon sequestration goals and contribute to biodiversity conservation and sustainability.
2. The new Forest and Biodiversity (FAB2) experiment uses native tree species in varying levels of species richness, phylogenetic diversity and functional diversity planted in 100 m^2 and 400 m^2 plots at 1 m spacing, appropriate for testing long-term ecosystem consequences. FAB2 was designed and established in conjunction with a prior experiment (FAB1) in which the same set of 12 species was planted in 16 m^2 plots at 0.5 m spacing. Both are adjacent to the BioDIV prairie-grassland diversity experiment, enabling comparative investigations of diversity and ecosystem function relationships between experimental grasslands and forests at different planting densities and plot sizes.
3. Within the first 6 years, mortality in 400 m^2 monoculture plots was higher than in 100 m^2 plots. The highest mortality occurred in *Tilia americana* and *Acer negundo* monocultures, but mortality for both species decreased with increasing plot diversity. These results demonstrate the importance of forest diversity in reducing mortality in some species and point to potential mechanisms, including light and drought stress, that cause tree mortality in vulnerable monocultures. The

experiment highlights challenges to maintaining monoculture and low-diversity treatments in tree mixture experiments of large extent.

4. FAB2 provides a long-term platform to test the mechanisms and processes that contribute to forest stability and ecosystem productivity in changing environments. Its ecosystem-scale design, and accompanying R package, are designed to discern species and lineage effects and multiple dimensions of diversity to inform restoration of ecosystem functions and services from forests. It also provides a platform for improving remote sensing approaches, including Uncrewed Aerial Vehicles (UAVs) equipped with LiDAR, multispectral and hyperspectral sensors, to complement ground-based monitoring. We aim for the experiment to contribute to international efforts to monitor and manage forests in the face of global change.

KEYWORDS
ecosystem function, experimental platform, functional diversity, phylogenetic diversity, remote sensing, tree diversity, tree genotype, tree mortality

1 | INTRODUCTION

In our era of rapid global change, restoring ecosystems based on sound ecological principles is critical (Leadley et al., 2022), making it essential to understand the drivers of ecosystem functions and services in the context of restoration (Díaz et al., 2013; Larkin et al., 2023). A rapidly growing research program aims to use well-designed experiments to test whether and how functional traits and diversity influence the provision of ecosystem services, including by forests (Paquette et al., 2018). Such large-scale experiments are critical for guiding forest restoration practices (Grossman et al., 2018), which will help support the goal set by the Kunming-Montreal Global Biodiversity Framework as part of the UN Convention on Biodiversity to provide guidelines and criteria for restoring degraded ecosystems (Leadley et al., 2022). These experiments also provide a platform for integrating remote sensing tools with measured biological processes on the ground and for advancing capabilities to monitor forest growth, diversity, and ecosystem function (Cavender-Bares et al., 2020, 2022; Williams et al., 2021). Such tools are urgently called for to advance the global monitoring of biodiversity and ecosystem function (Gonzalez et al., 2023).

Early forest plantation experiments in Poland, Denmark, and elsewhere demonstrated that species functional traits had clear effects on ecosystem function (Hobbie et al., 2006; Ladegaard-Pedersen et al., 2005; Reich et al., 2005; Vesterdal et al., 2008, 2013), while forest composition experiments in Costa Rica showed that greater functional diversity results in greater productivity (Ewel et al., 2015; Haggar & Ewel, 1997). These experiments were consistent with grassland experiments showing species effects (Hobbie, 1994, 1995; Wedin & Tilman, 1990) and diversity effects on productivity (Tilman, 1993, 1994; Eisenhauer et al., 2019, Hector et al., 1999). The herbaceous experiments, in particular, have demonstrated that above-ground processes, including those that can be remotely sensed, are linked to and predict below-ground processes (Cavender-Bares et al., 2021; Cline et al., 2018). Insights provided by studies from early experiments led to calls for a network of tree diversity experiments to determine whether these results would generalize to contrasting environmental conditions and biogeographic contexts (Verheyen et al., 2016). Other ecosystem functions beyond productivity, such as nutrient cycling, hydrologic processes and support for biodiversity at other trophic levels have received increasing attention, as has long-term ecosystem resilience. Experiments designed to test the effects of tree composition and diversity on multiple ecosystem functions and resilience have now emerged across the globe, led by TreeDivNet and the IDENT experiments (Paquette et al., 2018).

Results from newer forest diversity experiments have upheld previous findings that greater diversity contributes to higher productivity through a combination of complementarity and species effects (Grossman et al., 2017; Tobner et al., 2016; Williams et al., 2017). In addition, these new experiments have expanded on prior findings, showing that forest mixtures that include trees with different kinds of functional strategies can provide multiple ecosystem services (Messier et al., 2021) and improve resilience through reduced heterogeneity in survival and growth under drought (Hutchison et al., 2018). However, few of these experiments were designed to tease apart the consequences of different dimensions of diversity, including species richness, functional diversity and phylogenetic diversity. Of those that were, no prior experiments have large enough plots to test the consequences of these multiple dimensions of forest diversity for ecosystem functions with a large spatial footprint. Furthermore, no experiments that we know of can be used to compare biodiversity-ecosystem function (BEF) relationships between grasslands and forests that vary in plot size and planting density in the same soils and climate. The novelty of the FAB2 experimental platform lies in its potential to address these gaps.

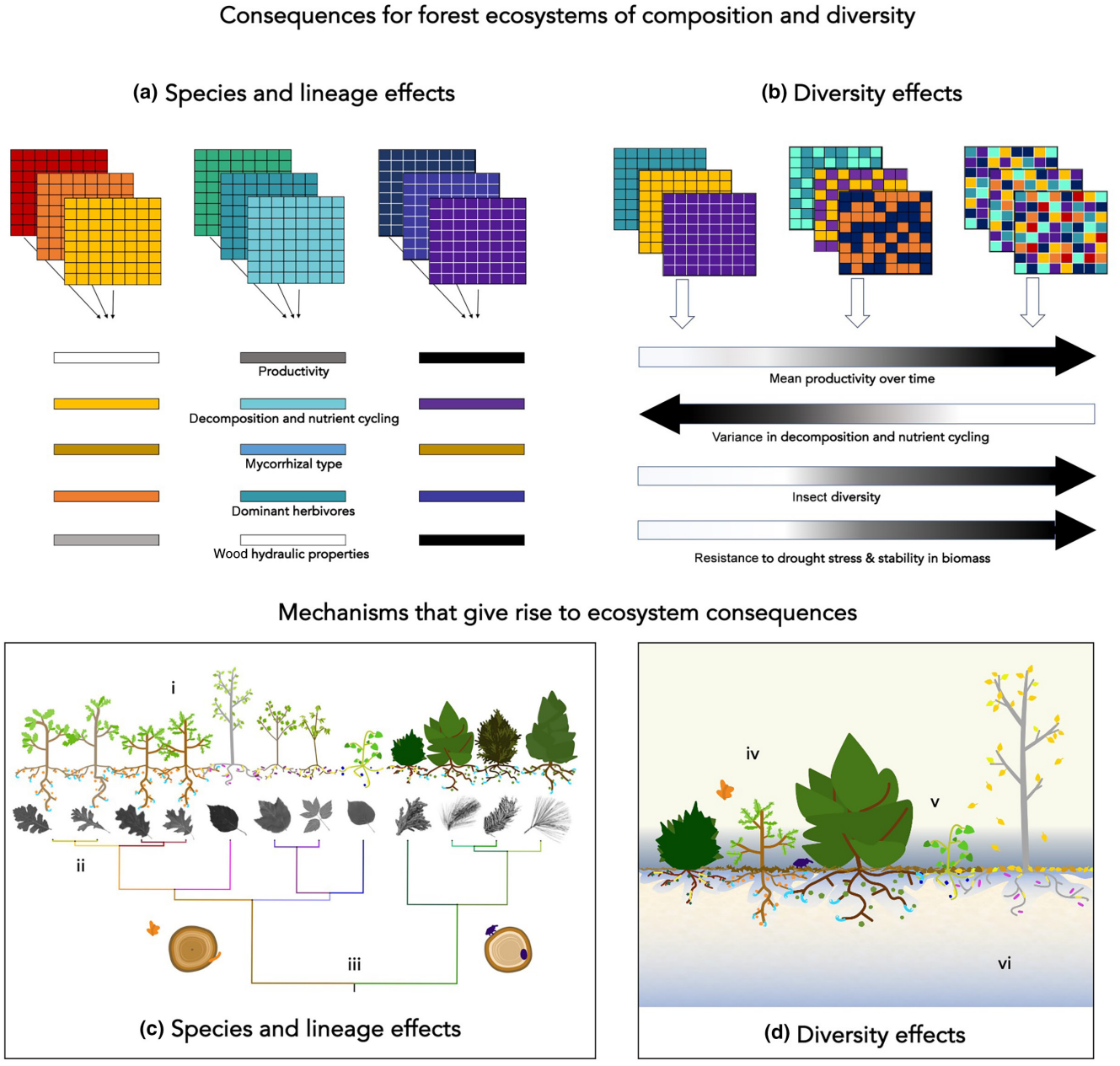
1.1 | Goals of the FAB2 experiment

We designed the FAB2 experiment to determine the influence of tree diversity and composition on long-term community processes, successional dynamics, ecosystem function and ecosystem resilience. We used native tree species that occur within the local region of the Cedar Creek Ecosystem Science Reserve that could plausibly co-occur in the absence of environmental filters. FAB2 differs from other tree diversity experiments in its potential to tease apart dimensions of forest diversity and in its large plot sizes. FAB2 was planted adjacent to and designed in conjunction with a smaller experiment FAB1 (Grossman et al., 2017), which has smaller plot sizes and higher planting density. Both experiments are adjacent to the long-term BioDIV grassland experiment (Figure 1). FAB2 is now the largest tree diversity experiment in North America, to our knowledge. The suite of Cedar Creek BEF experiments are the only in the world that enable direct comparisons of forest and grassland BEF relationships on the same soil and climate, with forest plots that differ in size (400, 100, 16 m^2) and planting density (1 and 0.5 m spacing).

Our main objectives in FAB2 are to test hypotheses that generate insights to aid forest restoration (i.e. improve degraded land to a state that resembles naturally assembled ecosystems, see Supporting Information 3), to observe long-term ecological succession and dynamics, and to test consequences of forest composition and diversity for ecosystem function. We are specifically interested in understanding the consequences of tree species and lineages (close relatives with a recent common ancestor) and of tree diversity for other trophic levels and for ecosystem functions, and in deciphering the causal mechanisms of these effects (Figure 2). We further seek to understand how plot size and planting density interact with composition and diversity to influence tree performance over time. Our guiding hypotheses for the experimental platform are developed in detail in Supporting Information 1 along with a series of definitions to provide clarity in Supporting Information 2.

The large plot sizes of FAB2, including very large monocultures, enable tests of species and lineage effects on ecosystem processes. Many such processes have an inherently large spatial footprint and may bleed into adjacent plots if the plot size is too small. FAB2 is also designed to test the importance of functional and phylogenetic diversity within a given species richness level. The similarity in composition and diversity treatments between FAB1 and FAB2 provides a unique opportunity to test whether the BEF relationships observed in FAB1 are supported in larger plots with wider spacing between trees. Large plot sizes also enable tests of whether identity and diversity effects increase through time, since they reduce bleeding effects that may be expected to increase as trees grow larger. For example, plant community diversity may influence soil microbial communities, both directly and as mediated by the tendency of plant diversity to enhance primary productivity. In experimental grasslands, more diverse communities tend to have higher microbial biomass, higher rates of soil respiration, and higher nitrogen mineralization rates, leading to higher nitrogen and carbon storage on decadal scales (Lange et al., 2023; Zak et al., 2003). Emerging experimental evidence from tree diversity experiments supports the role of plant diversity as a potential, though often indirect, driver of soil microbial activity (Beugnon et al., 2021; Bryant et al., in press) with deepening of plant-microbial linkages expected over time (Thakur et al., 2021). We anticipate that assemblages will vary in the quantity and quality of their inputs to soil, with consequences for the rates and magnitudes of soil microbial processes as well as how they change through time. Given that roots, leaf litter, and other sources of plant inputs may cross between nearby plots, we aimed to design

FIGURE 1 An aerial view of the Forest and Biodiversity (FAB2) experiment, showing the combination of 100 and 400 m^2 plots, adjacent to the smaller FAB1 experiment and the BioDiv grassland experiment in October 2023 at the Cedar Creek Ecosystem Science Reserve. Differences in colour and size of evergreen conifers and deciduous angiosperms in FAB2 are evident.



plots large enough to allow these processes and others (e.g. microclimate modulation, Atkins et al., 2023) to be studied while minimizing the influence of edge effects.

Given the large size of the experiment, we are developing remote sensing approaches for cost-effective long-term measurement of growth, survival, structure, phenology, and ecosystem processes. Our aim is to use both relatively inexpensive (multispectral, thermal, LiDAR) and more advanced (hyperspectral) sensors on UAV platforms to characterize canopy chemistry and changes in forest structure across the growing season and through succession in order to test our hypotheses. Remotely sensed information can be used to model and map canopy traits, volume, structure and ecosystem productivity that can be related to below-ground processes such as soil nutrient availability and soil processes.

The purpose of this article is to document the design, rationale and establishment history of the FAB2 long-term experimental platform and to test three specific hypotheses related to tree mortality in the early phases of the experiment. These hypotheses are linked to differences among species in vulnerability to light stress and drought, which may be influenced by diversity and exacerbated by plot size.

1. mortality is non-random with respect to identity and function, such that species or lineages are more vulnerable to light and drought stress will have higher mortality than others,
2. greater tree diversity reduces mortality and interacts with identity through changes in forest biomass and shade cover, such that mortality among vulnerable species is reduced in more diverse plots, and

FIGURE 2 The Forest and Biodiversity (FAB2) experiment is designed to test consequences of (a) tree species and lineages and of (b) multiple dimensions of forest diversity for ecosystem functions and for other trophic levels, and (c) to uncover the mechanisms underlying these effects. (a) The monocultures in FAB2 enable tests of species (and lineage) effects. Species vary in nutrient acquisition and use strategies, litter properties, wood and hydraulic properties, defence chemistry, symbiotic relationships, growth rates and resistance to stress—all of which may influence their fitness (response traits) as well as their effect on the environment around them (effect traits, sensu Lavorel & Garnier, 2002). We thus expect forest plots that differ in which species are planted to vary in productivity, microclimate, soil texture and pH, decomposition rates and carbon/nutrient cycling, dominant foliar herbivores, and soil fauna like worms, bacteria and fungi—including dominant mycorrizal types associated with tree roots. This conceptual figure illustrates the predicted influence on the environment of species belonging to one of three lineages, indicated by clusters of similar colours. Greater variation in ecosystem processes is expected among lineages that are phylogenetically or functionally dissimilar (plot colours are more distinct) than among closely related species that have more shared ancestry and/or species that are more functionally similar (plots colours are more similar). Hence, we expect closely related and/or functionally similar species to exhibit similar patterns in productivity, decomposition and carbon/nutrient cycling, dominant herbivores, mycorrhizal type and woody hydraulic properties important for productivity and resistance to drought. These hypotheses are illustrated with different coloured bars underneath the plots to indicate variation in the ecosystem and trophic-level consequences of different plant species and lineages. Grayscale bars indicate a gradient. (b) Through its nested dimensions of diversity (e.g. variation in functional and phylogenetic diversity within species richness levels), FAB2 also enables tests of forest diversity effects on above- and belowground productivity; herbivore composition, diversity and abundance, and their feedbacks to ecosystem productivity; and resistance and resilience of ecosystems through time. Various monocultures (left) bicultures of different phylogenetic and functional similarity (middle) and higher diversity treatments (right) are shown in (b) as examples to illustrate the consequences of tree diversity treatments, indicated below with directional arrows. With greater tree diversity, mean productivity is expected to increase, variance in decomposition and nutrient cycling is expected to decrease, local insect diversity is expected to increase and resistance to drought and biomass stability are expected to increase. (c and d) The replicated monocultures and range of mixtures provide a means to decipher potential roles of complementarity and selection effects as mechanisms by which diversity influences ecosystem functions and to examine how they may emerge from shifts in species interactions, including facilitation and niche partitioning. Included in the tree diversity experiment are twelve tree species native to Minnesota that span a wide range of lineages and functional traits. From left to right: *Quercus macrocarpa*, *Q. alba*, *Q. rubra*, *Q. ellipsoidalis*, *Betula papyrifera*, *Acer rubrum*, *A. negundo*, *Juniperus virginiana*, *Pinus resinosa*, *P. banksiana*, *P. strobus*. Some of the potential mechanisms underlying species and lineage effects associated with the experimental design are illustrated in (c), which shows foliar and wood traits differing among species and lineages with consequences for ecosystem processes and other trophic levels, including soil biota. The experimental design enables the study of (i) species differences in plant function and intrinsic growth rates, (ii) host specificity and co-evolutionary acquisition of symbionts, including bacterial and fungal partners, and (iii) the deep evolutionary divergence in wood and leaf structural properties and their consequences for ecosystem processes. (d) depicts some of the potential mechanisms underlying tree diversity effects that can be studied in the experiment. These effects include (iv) dilution effects, (v) phenological offsetting in light and nutrient use, and (vi) facilitation through shading and soil moisture maintenance. Detailed hypotheses and definitions are provided in the Supporting Information.

3. ecosystem extent influences vulnerability to stress, such that mortality of vulnerable species is greater in larger plot sizes where light stress is exacerbated.

2 | METHODS

2.1 | The Forest and Biodiversity (FAB2) experiment

FAB2 was established in an abandoned old field dominated by herbaceous species at the Cedar Creek Ecosystem Science Reserve, a 2300 ha reserve and National Science Foundation Long Term Ecological Research site in eastern Minnesota, USA (45°25′ N, 93°10′ W). The site is situated on excessively drained sandy soils (Lamellic Udipsamments, Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, 2024) of the Anoka Sand Plain and has a humid continental climate with warm summers and cold winters. Cedar Creek is located at the boundary of the Midwestern tallgrass prairies, Eastern deciduous forests and Northern boreal forests. In 2016, we established the first phase of FAB2, which involved planting trees in a 1 m grid into 100 m^2 plots, including replicated monocultures and two-species, four-species, six-species and 12-species polycultures (Table 1, Table S1). In 2017, we established the second phase, which involved planting trees in a 1 m grid into 400 m^2 plots, including replicated monocultures and five 12-species polycultures. The full experiment covers approximately 6.5 ha (Figure 1). In the year prior to planting phase 1, the experimental site was treated by removing nearby trees and stumps that could cast shade, tilling the topsoil and burning the herbaceous vegetation. The experiment was fenced to exclude large mammalian herbivores. We mowed between rows of planted trees and occasionally hand-weeded 2–3 times during the growing season each year during the first 3 years, and at least once per year in subsequent years to minimize competition from herbaceous vegetation. Trees were irrigated in spring and summer in the first 6 years of the experiment (2016–2022) using two Kifco T200L water reels. In 2023, trees were irrigated using sprayers only during periods with lower precipitation than average. Trees are no longer irrigated after canopy closure, which has already occurred for many assemblages. From 2016 to 2022, *Spermophilus tridecemlineatus* (gophers) were monitored and trapped every year to minimize disturbance. The field experiment was established with approval from the Cedar Creek Ecosystem Science Reserve as experiment ID 291.

No. plots	Species richness	Description	Size
36	1	12 monocultures replicated 3×	100 m^2
47	2	11 random; 36 selected for different PV-FV combinations	100 m^2
45	4	9 random; 36 selected for different PV-FV combinations	100 m^2
10	6	Random	100 m^2
10	12	All species polycultures	100 m^2
36* (25)	1	Monocultures	400 m^2
5	12	All species polycultures	400 m^2
30	1, 2, 4	Oak-DIV	100 m^2

*There were originally 36 400 m^2 monoculture plots but only 25 of those remain, due to mortality. The Oak-DIV experiment, nested within the larger experiment, includes 30 plots in total, including three monocultures of each of the four oak species plus three replicates of all of the two and four species combinations. Some of the Oak-DIV plots are also part of the general FAB2 experiment.

TABLE 1 General design of FAB2 with the number of plots in each species richness level, the description of the treatments and how they were selected, and the size of the plots.

2.2 | Tree diversity gradient across functional and phylogenetic variability levels in 100 m^2 plots

The main part of the FAB2 experiment includes 148 plots each of 10×10 m (100 m^2) planted in species richness levels of 1, 2, 4, 6 or 12. Of these, 36 plots were monocultures (12 species each replicated in three monocultures), 47 plots were bicultures, 45 plots were four-species mixtures, 10 plots were six-species mixtures and 10 plots were 12-species mixtures. Among the bicultures, 11 were combinations of randomly selected species and 36 were bicultures of species selected to achieve targeted phylogenetic variability (PV)—functional variability (FV) combinations, also calculated in terms of phylogenetic diversity (PD)—functional diversity (FD) (details below). Among the four-species mixtures, nine were combinations of randomly selected species, and 36 were mixtures of species selected based on achieving targeted PV–FV combinations. The six-species mixtures were all created through random selection of species. Survival was approximately 95% per year on average across all species (Table 3). All seedlings that died were replanted each year between 2017 and 2020.

2.3 | Selection of native tree species

Prior to designing both FAB experiments, we planted 22 native tree species into an old field at CCESR without watering or other amendments. For FAB1 and FAB2, we chose only species that had survival rates higher than 50% in the initial planting. Our final planting list of 12 native species for the FAB experiments included eight angiosperms, including four oak species (*Q. alba*, *Q. ellipsoidalis*, *Q. macrocarpa* and *Q. rubra*), a birch (*B. papyrifera*), two maple species (*A. rubrum* and *A. negundo*), and basswood (*Tilia americana*), as well as four conifers, including three pines (*Pinus banksiana*, *P. resinosa* and *P. strobus*) and eastern red cedar (*Juniperus virginiana*).

2.4 | Tracking maternal families for five species

To track genetic identities within populations and account for genetic variation within some of the species, individuals from half-sib families were planted for five species—*Acer rubrum*, *Betula papyrifera*, *Quercus ellipsoidalis*, *Q. macrocarpa* and *Q. rubra*. Seeds were collected from known and geolocated mother trees in Minnesota (Figure S1), germinated and grown in the PRT Dryden nursery in Dryden, ON, Canada (www.prt.com), and returned to Minnesota after 1 year of growth. These individuals were randomly assigned to different treatments in which the species were represented (Table 2).

2.5 | Large 400 m^2 monocultures and polycultures

Large monocultures and 12-species polycultures in 20×20 m plots (400 m^2) were planted in an arrangement that interspersed them throughout the experimental area, with 1 m spacing between trees (Figure 1). The rationale for these larger plot sizes was to reduce edge effects, blown-in litter and root in-growth from neighbouring assemblages to test for long-term ecosystem effects that might not emerge in smaller plots. Originally, all 12 species were planted in large monocultures replicated 3× along with five 12-species polycultures. In spring 2022, due to low initial survival for *Tilia americana*, *Acer negundo*, *Quercus ellipsoidalis*, *Q. alba* and *Betula papyrifera* in 400 m^2 monocultures, eleven 400 m^2 monoculture plots were removed from the experiment (Table 2) and converted to 10×10 m plots to create the Oak-DIV experiment, which compares the interspecific interactions of oak species from the same and different lineages (*Quercus* section *Quercus*, white oaks vs. *Quercus* section *Lobatae*, red oaks). No large monocultures remained for *T. americana*. One large monoculture was retained for *B. papyrifera*, *Q. alba* and *Q. rubra*, and two for *A. negundo* (Table 2).

TABLE 2 Scientific and common names of species and taxonomic families included in FAB2.

Species	Family	Common name	Maternal lines tracked	100 m^2 monocultures	400 m^2 monocultures
Acer negundo	Sapindaceae	Box elder		3	3
Acer rubrum	Sapindaceae	Red maple	20	3	3
Betula papyrifera	Betulaceae	Paper birch	21	3	0
Juniperus virginiana	Cupressaceae	Red cedar		3	3
Pinus banksiana	Pinaceae	Jack pine		3	3
Pinus strobus	Pinaceae	White pine		3	3
Pinus resinosa	Pinaceae	Red pine		3	3
Quercus alba	Fagaceae	White oak		3	1
Quercus ellipsoidalis	Fagaceae	Northern pin oak	13	3	3
Quercus macrocarpa	Fagaceae	Bur oak	12	3	3
Quercus rubra	Fagaceae	Red oak	18	3	1
Tilia americana	Malvaceae	American basswood		5 ($3 \times 100\,m^2 + ½$ of 400 m^2 plot)	0

Note: The number of maternal families raised for inclusion and tracking in the 100 m^2 experimental plots are shown for each of the five tree species for which we collected our own seed stock. The number of monocultures for each species for the two plot sizes is shown.

2.6 | Phylogenetic and functional variability and diversity

The mixture of close relatives and distant relatives with a range of trait combinations allowed us to create mixtures of (1) low phylogenetic and functional variability, (2) high phylogenetic and functional variability, (3) low phylogenetic and high functional variability, and (4) high phylogenetic and low functional variability. These combinations were previously tested in two species mixtures in FAB1 (Grossman et al., 2017) and the long-term consequences of these interactions are still being tested (Bryant et al., in press). In the FAB2 experiment, we created more combinations by creating two-, four- and six-species mixtures (Table 1).

During the design phase, phylogenetic variability and diversity were calculated based on the phylogenetic tree published by Zanne et al. (2014) with modifications. We calculated phylogenetic species variability (Helmus, 2007) in Picante (Kembel et al., 2010), which we call phylogenetic variability (PV) for simplicity, and phylogenetic diversity (PD) based on Faith's PD. PV varies between 0 and 1 and is independent of species richness. After planting, we recalculated these metrics using the Smith & Brown, 2018 v.01 megaphylogeny (Smith & Brown, 2018), pruned to the 12 FAB species and ultrametricized using phytools (Revell, 2012). Both pruned trees are shown in Figure S3.

We calculated functional variability (FV) and functional diversity (FD) from the same metrics using a multivariate trait dendrogram treated as a bifurcating phylogeny (Cadotte et al., 2009; Cavender-Bares & Reich, 2012). We scaled each trait a mean of 0 and SD of 1 and used the hclust algorithm in R to create a trait dendrogram and cophenetic distance matrix. We chose this method of calculating functional diversity to be directly comparable with the method of calculating phylogenetic diversity. During the design phase, functional variability and diversity were based on species-level measured values of leaf mass per unit area (LMA) and leaf nitrogen concentration (N_{mass}) from the GLOPNET database (Wright et al., 2004), ranked values of wood density, shade tolerance and drought tolerance based on the authors' expert knowledge of the trees, and binary values of leaf habit (evergreen/deciduous), mycorrhizal type (ECM or AM) and calcium use (high/low). We recalculated functional variability and diversity here using measured values of LMA, N_{mass} (Sendall & Reich, 2013; Wright et al., 2004) and wood density (Jenkins et al., 2003), binary values of leaf habit, mycorrhizal type (Averill et al., 2019) and calcium use, and indices of shade and drought tolerances (Niinemets & Valladares, 2006). We chose these traits to encompass functions related to nutrient use—including micronutrients important for soil processes and soil organisms—as well as water use and drought tolerance, and light use and shade tolerance. These traits are linked to the major axes of environmental variation important for niche partitioning in natural forest communities of the region.

Within each species richness level, PV was binned into eight quantiles and compared to FV. Species pairs or four-species mixtures were randomly drawn from each quantile in a manner that would create low PV-high FV, low FV-high FV, high PV-high FV and low PV-low FV combinations, to the extent possible. These values are provided for each plot in Table S1. The range of variation on these two axes is shown in Figure S2 for the four-species mixtures. We also recalculated PD and FD (rooted and unrooted, Faith, 1992) using the updated input data described above (Supporting Information). The spatial arrangement of PV and FV plots is visualized in Figure 3.

2.7 | Data analysis

We fit linear models to the tree mortality data (Cavender-Bares et al., 2024) to test for the effects of plot diversity and composition, species and plot size. Percent mortality was calculated for

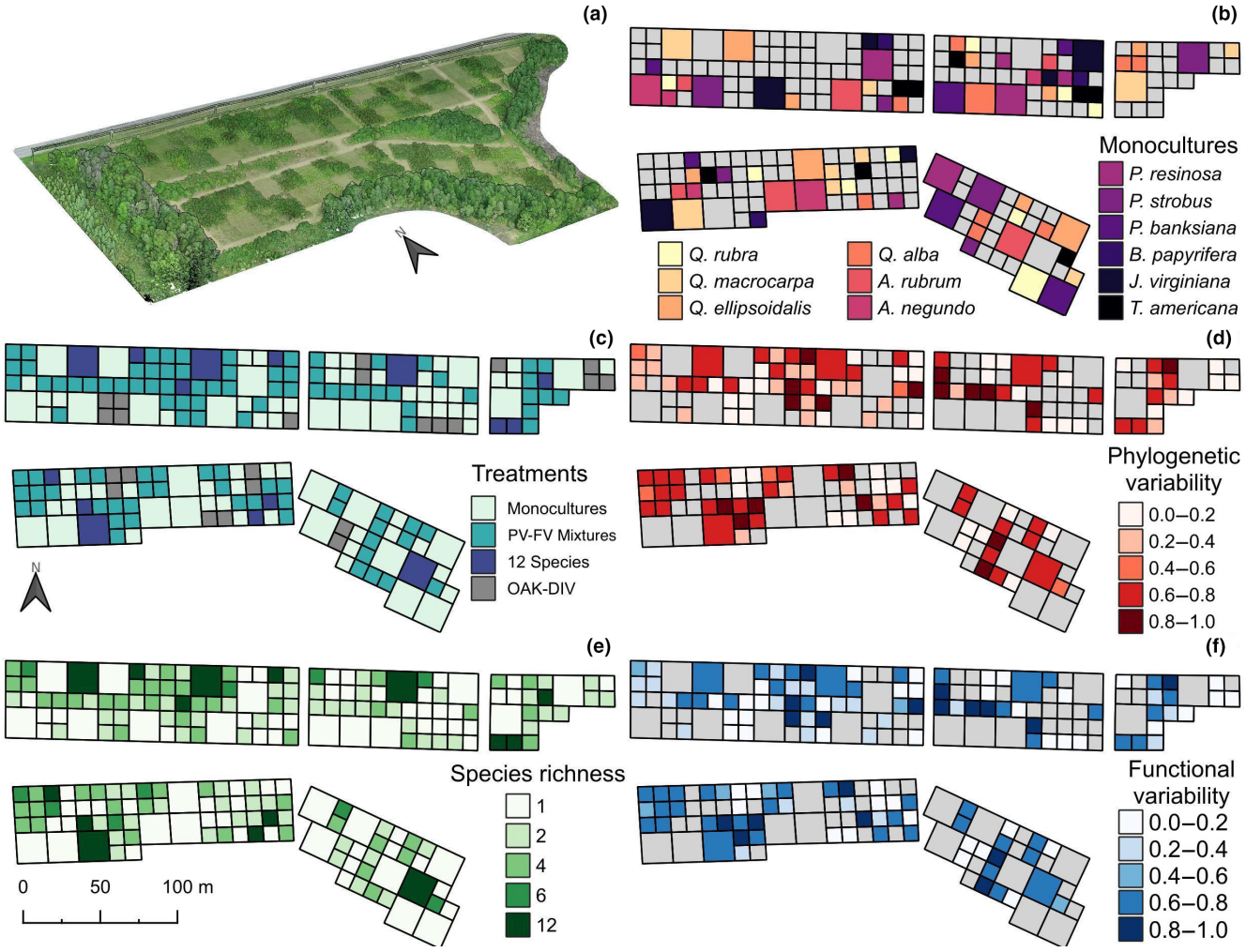


FIGURE 3 Experimental design of the Forest and Biodiversity Experiment 2 at the Cedar Creek Ecosystem Science Reserve. (a) A LiDAR image during summer 2022. Colour images show the spatial arrangement of (b) species composition of plots with monocultures, (c) monocultures, PV-FV mixtures, 12 species mixtures and the oak species mixtures within the Oak-DIV nested experiment, (d) phylogenetic variability within plots, (e) number of species in each plot and (f) functional variability within plots.

each species in each plot where it occurred for every year. We ran all models as untransformed percent mortality as well as arcsine transformed values. In the first set of analyses using only 100 m^2 plots, percent mortality was treated as the independent variable. Species, plot diversity and their interaction were the predictor variables. In five separate analyses, species richness, functional diversity, functional variability, phylogenetic diversity or phylogenetic variability were treated as the diversity variable. In a second set of analyses, using only 400 m^2 plots, percent mortality was treated as the independent variable predicted by species, plot diversity and their interaction. Again, species richness, phylogenetic variability, phylogenetic diversity, functional variability, or functional diversity were each treated in turn as the diversity variable. In a third set of analyses, both 100 and 400 m^2 plots were included. Percent mortality was treated as the independent variable, and the predictor variables were plot size, species and plot diversity, as well as the interactions of species with plot size and with plot diversity. Finally, a last analysis using both 100 and 400 m^2 plots treated percent mortality as the independent variable and treated as predictor variables species, plot size, the proportion of angiosperms in the plot and the interactions of species with plot size and with proportion of angiosperms in the plot. There was no substantial difference in results when percent mortality or arcsine transformed mortality values were used, indicating that the model results are robust to normality assumptions. Model results using transformed data are reported in the text, but untransformed data are used for the figures. Linear models were run in JMP version 16.0 (SAS Institute Inc., Cary, NC, 1989–2023).

2.8 | R package for FAB2 mapping and analysis

We have formalized our scripts related to our hypothesis into an open-source R software package called FAB (Guzmán & Cavender-Bares, 2024). The FAB R package is available on GitHub and provides tools to process and visualize data from inventories derived from Forest and Biodiversity Experiments. This package includes functions for estimating the annual mortality of individuals and species within plots,

excluding edge rows of trees in plots, splitting large plots into smaller ones, estimating diversity metrics (as used in the manuscript), and calculating Net Biodiversity Effects (NBE), including complementarity and selection effects according to the method of Loreau and Hector (2001).

2.9 | Measurements

Soils (1 composite of 10 cores/100 m^2 plot and 20 cores/400 m^2 plot) from 3 depths (0–15, 15–30, 30–60 cm) were archived at the start of the experiment. All trees are measured annually for tree survival and growth, measured as height, stem basal diameter and diameter at breast height once it is reached. We also collected multispectral imagery and LiDAR point clouds over the experiment monthly throughout the growing season starting in 2022. Herbivore and disease monitoring will take place both opportunistically (e.g., as infestations or outbreaks occur) and through periodic (one in ~5 year) stratified random surveys of end-of-season (August/September) generalist herbivory (leaf chewing and skeletonizing intensity), specialist herbivory (gall and leaf miner abundance), and accumulated

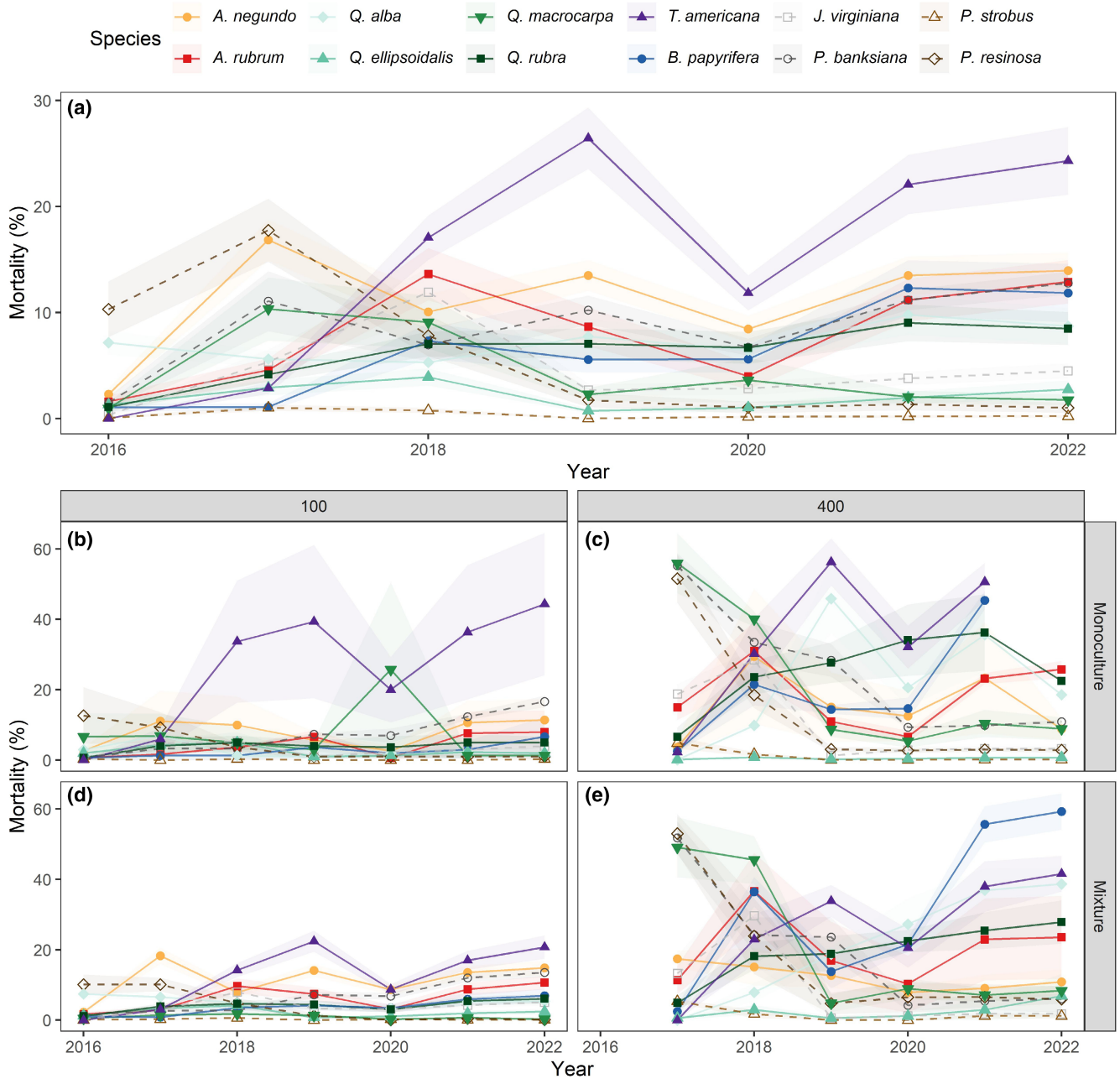


FIGURE 4 Percent mortality of trees. (a) average mortality by species in all plots. Mortality by species in (b) 100 m^2 monoculture plots (c), 400 m^2 monocultures, (d) 100 m^2 mixtures (any plot with more than one species), e) 400 m^2 mixtures. Mean values per species are shown for each year, with standard error confidence intervals. Mortality percentages were calculated to include any trees newly or previously planted in the plots. The 100 m^2 plots were planted in 2016, the 400 m^2 plots were planted in 2017; these were replanted as necessary for 3 years.

pathogen load (foliar fungal damage intensity). Following attainment of reproductive maturity for most species (~10+ years following establishment), we will also conduct regular stratified random surveys of seed output across diversity treatments.

3 | RESULTS

Mortality rates of trees (Figure 4) differed among species (DF=11, SS=5.69, F ratio=39.46, $p<0.0001$) and with plot size (DF=1, SS=6.16, F ratio=470, $p<0.0001$). Across all species, mortality averaged 5% per year in the 100 m^2 plots and 17% per year in the 400 m^2 plots (Table 3). Species also differed in their response to plot size as indicated by the significant interaction (DF=11, SS=2.9, F ratio=20.1, $p<0.0001$) when data for both plot sizes were analysed together (Table S4).

Within the 100 m^2 plots, mortality of trees significantly differed between species (DF=11, SS=4.85, F ratio=44.45, $p<0.0001$), and across diversity treatments (DF=1, SS=0.102, F ratio=10.34, $p=0.0013$ for PD) with a significant species by diversity interaction (DF=11, SS=0.32, F ratio=2.93, $p=0.0007$ for PD) and lower overall mortality in more diverse plots. Species, plot diversity and their interaction significantly predicted mortality, regardless of which diversity metric was used (Table S4). *Tilia americana* and *Acer negundo* in particular showed decreasing mortality with increasing plot diversity (Figure 5a–c). Plot composition in conjunction with diversity was also important in predicting mortality. The highest mortality occurred in low diversity angiosperm mixtures (Figure 5e,f). Only lower diversity mixtures were composed of angiosperms only. The proportion of angiosperms in a mixture significantly predicted percent tree mortality (DF=1, SS=0.08, F ratio=9.5, $p=0.0021$) when species, phylogenetic

TABLE 3 Average tree mortality rates (%) by species and year across all 100 m^2 assemblages (top) and 400 m^2 assemblages (bottom).

	% mortality								
Species	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**	**Average**	**SD**
100 m^2 plots									
Acer negundo	2.8	4.2	9.1	13.0	8.1	13.9	15.0	9.4	3.8
Acer rubrum	1.7	0.5	8.3	7.6	2.4	8.5	10.0	5.6	3.5
Betula papyrifera	0.8	0.3	3.0	4.3	3.1	5.5	6.8	3.4	2.1
Juniperus virginiana	0.5	1.5	8.5	3.1	2.9	3.7	4.8	3.6	2.2
Quercus alba	6.3	2.2	4.7	4.0	3.0	4.1	6.7	4.4	1.4
Quercus ellipsoidalis	1.5	1.3	3.6	0.8	1.0	1.6	3.9	2.0	1.2
Quercus macrocarpa	2.2	2.1	2.5	1.3	0.5	0.9	6.3	2.2	1.9
Quercus rubra	1.1	1.3	5.4	4.0	3.2	5.3	9.8	4.3	2.6
Pinus banksiana	1.9	0.9	3.6	7.7	7.5	12.4	14.5	6.9	4.7
Pinus resinosa	12.5	3.3	4.8	1.1	0.4	0.5	0.5	3.3	1.8
Pinus strobus	0.2	0.0	0.9	0.0	0.2	0.2	0.2	0.2	0.3
Tilia americana	2.5	1.7	20.1	27.8	12.5	23.2	28.0	16.5	9.3
Average	2.8	1.6	6.2	6.2	3.7	6.6	8.9	5.2	
SD	3.4	1.2	5.1	7.7	3.8	6.9	7.6	4.3	
400 m^2 plots									
Acer negundo		7.7	27.7	14.9	12.0	21.9	9.2	15.6	7
Acer rubrum		17.6	33.0	11.8	7.2	23.3	25.8	19.8	9
Betula papyrifera		2.6	24.4	14.6	15.8	47.2	59.9	27.4	20
Juniperus virginiana		19.6	29.5	1.3	2.7	3.1	3.2	9.9	11
Quercus alba		0.1	9.6	38.1	20.7	33.7	24.9	21.2	13
Quercus ellipsoidalis		0.2	1.1	0.3	0.5	0.9	1.5	0.8	0
Quercus macrocarpa		55.1	42.8	8.8	5.9	10.0	9.2	22.0	19
Quercus rubra		5.9	22.6	22.6	29.2	31.4	24.2	22.7	8
Pinus banksiana		55.3	33.7	27.8	8.8	9.7	10.6	24.3	17
Pinus resinosa		51.8	19.6	3.4	3.2	3.4	3.2	14.1	18
Pinus strobus		4.9	1.8	0.1	0.1	0.3	0.3	1.2	2
Tilia americana		1.7	29.6	40.8	23.4	41.2	42.2	29.8	14
Average		18.5	23.0	15.4	10.8	18.9	17.9	17.4	
SD		22.3	12.9	14.2	9.6	16.6	18.4	9.4	

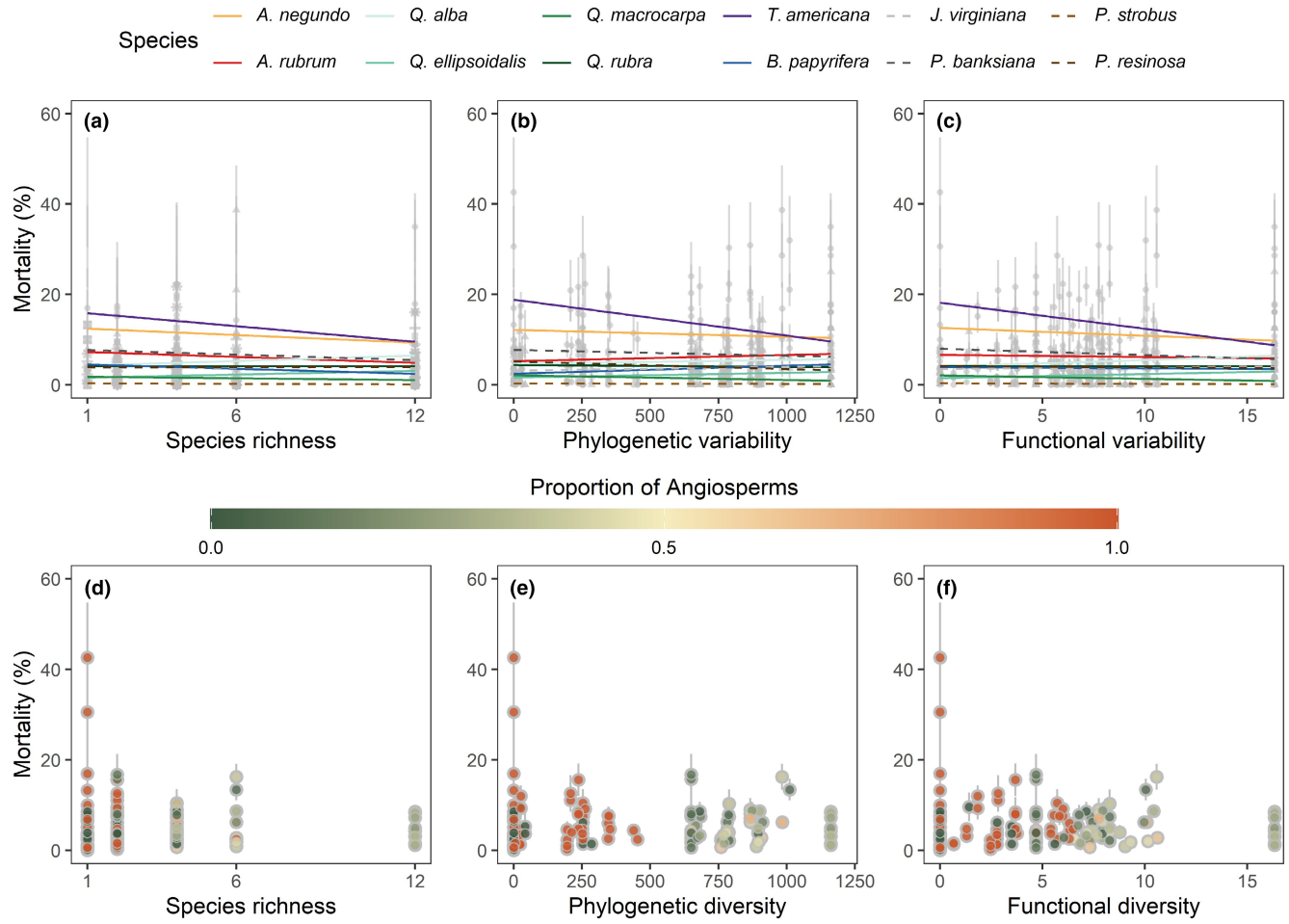


FIGURE 5 Tree mortality by species or plot composition in relation to plot diversity. In the top three panels, percent mortality per species averaged over time is shown in relation to (a) species richness level, (b) phylogenetic diversity, calculated as the sum of the phylogenetic branch lengths of all species in the assemblage (Faith's PD) with monocultures shown as zero phylogenetic diversity and (c) functional diversity calculated as the sum of functional distances for a suite of eight traits, with monocultures shown as zero functional diversity. Linear models are fit to each species, shown as different coloured lines. Solid lines are angiosperms and dashed lines are gymnosperms. In the bottom three panels, mean percent mortality by assemblage averaged across time is shown in relation to (d) species richness, (e) phylogenetic diversity and (f) functional diversity. The proportion of angiosperm tree species in each plot is colour indicated, with increasing proportion of angiosperms shown as more orange and greater proportion of conifers as more green.

diversity (or other metric of diversity) and the interactions with species were included in the model. When phylogenetic diversity (or other metric of diversity) was not included in the model, the proportion of angiosperms was not directly significant in predicting tree mortality but the interaction of species and proportion of angiosperms in the mixture was significant (DF = 11, SS = 0.295, F ratio = 3.1, $p = 0.0004$, Table S4). When only the 400 m^2 plots were included in the analysis, species significantly differed in mortality (DF = 11, SS = 3.79, F ratio = 39.91, $p < 0.0001$, Figure 4, Table S4), but no diversity effect was discernable. Given these comprise 36 monoculture plots and only five 12-species plots, there is limited power to detect diversity effects.

4 | DISCUSSION

We present first results of a major long-term tree diversity experiment (FAB2) that varies multiple dimensions of forest diversity to provide guidance on forest restoration in accordance with the Kunming-Montreal UN Global Biodiversity Framework. The adjacency of the experiment to the prior FAB1 experiment with smaller plot sizes and higher planting density and to the BioDIV grassland experiment (Figure 1) enables long-term comparison of biodiversity-ecosystem function relationships and the potential to decipher underlying mechanisms. Establishment of this kind of experimental platform requires many people and years of sustained effort and resource inputs—a challenge well-suited to the NSF Long-Term Ecological Research program that Cedar Creek Ecosystem Science Reserve is part of. The design is accompanied by a conceptual framework (Figure 2) and series of hypotheses (Supporting Information 2) and definitions (Supporting Information 3), aimed at guiding research and researchers using the platform. To facilitate data analysis of the experiment, we have developed an R package for the FAB experiments, which includes data used in the design. In addition to presenting the experimental

design the guiding conceptual framework, and the analysis tool, we document initial mortality results and explain the major challenges we faced in establishing the experiment.

In the initial period of the experiment, mortality of trees significantly differed among species, by diversity treatment, and with plot size, providing support for our specific hypotheses. The highest mortality occurred in low diversity broadleaf (angiosperm) plots. Despite irrigation, it is likely that multiple years of successive drought caused greater mortality in angiosperm monocultures (Figure 5), where lack of shading by fast-growing conifer neighbours may have caused excessive evaporative loss. As an example of species-specific vulnerability, *Tilia americana* had high levels of mortality in both plot sizes, particularly in monocultures and in low diversity angiosperm mixtures. We conjecture that this outcome resulted from its dependence on shade from heterospecific neighbours, given that its growth in FAB1 was enhanced by shading (Kothari et al., 2021). *Acer negundo* showed a similar pattern, and these two species are the most shade-tolerant in the experiment (Niinemets & Valladares, 2006). These results point to the importance of diversity effects in preventing mortality of vulnerable tree species at the seedling stage.

The large variation in functional and phylogenetic composition across species richness levels creates an important foundation to test critical hypotheses about the consequences of species and lineages and their interactions for ecosystems and other trophic levels. The lessons that we stand to learn from this major experiment, in concert with similar experiments established globally (Paquette et al., 2018), will provide critical insights that can inform ecosystem management on our rapidly changing planet. Nevertheless, establishing and maintaining a large manipulative tree diversity experiment poses a number of challenges. We faced several issues in designing the experiment. The 12 species in the experiment were chosen primarily based on their survival rate in a preliminary (pre-FAB) planting experiment at Cedar Creek Ecosystem Science Reserve without watering, weeding or fertilizer amendments. With this species pool, it was not possible to create diversity treatments where FV and PV or FD and PD were fully orthogonal. Different ways of calculating phylogenetic and functional diversity also give different spreads of data. The high mortality during establishment of the experiment required us to replant trees from 2017 to 2020 each year in the 100 m^2 plots and from 2018 to –2022 in the 400 m^2 plots. In 2022, we had shortfalls in tree availability for replanting the 400 m^2 plots due to the COVID-19 pandemic. These trees were replaced in spring 2023, the last year of replanting. In 2022, Cedar Creek experienced an extreme summer drought, and some new plantings were especially susceptible to these conditions. Finally, the large size of the experiment makes exhaustive measurements of all individual trees beyond the annual growth survey challenging, and subsampling is critical. We are increasingly relying on remotely sensed measures of trees and plots to complement subsampling on the ground (Figure 3).

FAB2 provides a means to investigate the connections between identity, multiple dimensions of forest diversity, ecosystem functions, and biodiversity at other trophic levels. Our hope is that by providing a platform to test the consequences and mechanisms of relationships between forest diversity and ecosystem function, we will inform efforts to restore degraded or once-forested land into ecosystems that can sustain functions critical to our life support systems. These ecosystem services include climate regulation, biogeochemical and hydrologic cycling, air pollution removal, and support of many forms of biodiversity above- and below-ground. Such human-led efforts will be increasing essential to maintaining a habitable planet for humanity in our era of rapid global change.

AUTHOR CONTRIBUTIONS

Jeannine Cavender-Bares, Rebecca A. Montgomery and Sarah E. Hobbie conceived of the experiment. Jeannine Cavender-Bares designed the experiment with input from Sarah E. Hobbie and Rebecca A. Montgomery. Cathleen N. Lapadat led the establishment, maintenance and monitoring of the experiment. Matthew A. Kaproth led the seed collection and establishment of five species for genotype tracking. Jeannine Cavender-Bares wrote the initial manuscript with Cathleen N. Lapadat, Jake J. Grossman, Shan Kothari, Sarah E. Hobbie and Matthew A. Kaproth. All authors contributed intellectually to the project and to editing the manuscript. Jeannine Cavender-Bares, Cathleen N. Lapadat and José Antonio Guzmán Quesada analysed the data. José Antonio Guzmán Quesada, Jeannine Cavender-Bares, Maria Park and Matthew A. Kaproth prepared the figures with input from Jake J. Grossman, Sarah E. Hobbie and Shan Kothari. José Antonio Guzmán Quesada led the development of the R package with feedback from Jeannine Cavender-Bares.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/2041-210X.14435.

DATA AVAILABILITY STATEMENT

Data available via: https://portal.edirepository.org/nis/mapbrowse?scope=knb-lter-cdr&identifier=737&revision=2, https://doi.org/10.6073/pasta/9dd4a62f134757068a7d0a2992ef4593 (Cavender-Bares

et al., 2024). The R package developed for the forest and biodiversity experiments (i.e., FAB R package) is available at GitHub at (https://github.com/Cavender-Bares-Lab/FAB) and archived at Zenodo under version 0.1 https://doi.org/10.5281/zenodo.13800914 (Guzmán & Cavender-Bares, 2024).

STATEMENT OF INCLUSION

We acknowledge that the FAB2 experiment was established on Dakota and Ojibwe land. Our study brings together contributors from diverse backgrounds representing diversity in gender, age, sexual orientation, race and ethnicity and nationality. All authors were engaged early on with the manuscript preparation to ensure that the diverse sets of perspectives they represent was considered from the onset. The authors all work or have worked at Cedar Creek Ecosystem Science Reserve and are dedicated—through the processes of research, conservation, education and public engagement—to inclusion and bridging the gaps between science, community and government.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Supporting Information 1. Hypotheses

Supporting Information 2. Definitions

Supporting Information 3. Plot information, Table S1

Figure S1. For five species, seeds were collected from known mother trees within 100 km of the Cedar Creek Ecosystem Science Reserve (CCESR), East Bethel, MN, USA.

Figure S2. Possible treatment combinations based on functional variability (FV) and phylogenetic variability (PV) of tree species for (A) two species and (B) four species mixtures. For the four species mixtures, the PV-FV selected mixtures are shown in blue squares (B), and the randomly drawn combinations of four species are shown in red circles (C).

Figure S3. Original phylogeny from design phase (A) and current phylogeny (B) used for calculating phylogenetic variability and diversity.

Figure S4. Linear model results. In all analyses, percent mortality (calculated per species in every plot where it was planted) was treated as the independent variable.

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