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Positive feedbacks maintain alternative stable states of arbuscular vs. ectomycorrhizal forests

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Abstract: Most trees on Earth form a symbiosis with either arbuscular or ectomycorrhizal fungi (1). By forming common mycorrhizal networks and actively modifying the soil environment, these contrasting symbioses may generate positive feedbacks that favor their own mycorrhizal strategy at the expense of the alternative strategy (2, 3). Here we use continental scale forest inventory data to show arbuscular and ectomycorrhizal tree recruitment and mortality exhibit positive con-mycorrhizal density dependence, and data-driven simulations show these positive feedbacks are sufficient in magnitude to generate and maintain alternative stable states of the forest mycobiome. Given the links between forest mycorrhizal strategy and carbon sequestration potential (1, 5, 6), the presence of mycorrhizal-mediated alternative stable states affects how we forecast forest composition, carbon sequestration and terrestrial climate feedbacks.

Most trees on Earth form belowground networks with symbiotic mycorrhizal fungi, which in turn can connect different tree species within a forest (7). Yet, the vast majority of tree species can only form one of two predominant functional types of mycorrhizal symbiosis, arbuscular or ectomycorrhizal (AM or EM), restricting which tree species may be connected to which other tree species within a symbiotic network. Common mycorrhizal networks may facilitate resource transfers which favor the recruitment and survival of their own strategy (the con-mycorrhizal strategy) at the expense of the alternative strategy (8–11). Furthermore, once established AM and EM forests may engineer soil nutrient cycling conditions which further reinforce positive con-mycorrhizal feedbacks, favoring their own mycorrhizal strategy (2, 12–14). If such positive feedbacks exist across a wide range of forests they may, in time, generate alternative stable states of forested biomes, mediated by interactions with the forest fungal microbiome (4). As a result, forest mycorrhizal type may act as a hierarchical filter on forest biodiversity, restricting community membership as a function of mycorrhizal compatibility, which may be further reinforced by soil nutrient cycle feedbacks. Positive con-mycorrhizal feedbacks may still allow persistence of a diverse forest, while simultaneously driving mycorrhizal mono-dominance at the functional group level, and alternative stable states of the forest mycobioime at large spatial scales. Given the links between forest mycorrhizal status and carbon sequestration potential (1, 5, 6), the presence of mycorrhiza-mediated alternate stable states would affect how we forecast forest composition and emergent ecosystem carbon balance.

The potential for AM and EM symbioses to shape the composition of forests at continental scale was originally hypothesized three decades ago (15). More recent work lends support to this idea, showing a clear bimodality of AM vs. EM forest dominance, where mixed mycorrhizal forests are far rarer than expected by chance (16). While this co-occurrence pattern is consistent with the presence of mycorrhizal alternative stable states, there remain numerous other possible explanations for this pattern (17), particularly environmental filtering and dispersal limitation (18, 19), which can also lead to the clumping of similar species (19, 20). Demonstrating that these patterns reflect mycorrhizal-induced alternative stable states requires evidence of (i) bimodality in the frequency distribution of forest mycorrhizal types, (ii) positive community feedbacks in

time that could give rise to alternative stable states, and (iii) that observed feedbacks are strong enough to generate and maintain alternative stable states in the face of environmental heterogeneity and demographic stochasticity (21). Furthermore, each of these points must account for potential non-linear controls of the environment, and signatures of dispersal limitation (1, 19). Given the importance of these symbiotic forest types for governing elemental cycling across the global forest system, identifying these mechanisms is critical for accurately projecting carbon and nutrient dynamics under current and future climate change scenarios.

Using long-term forest inventory data across the United States, we show clear bimodality in AM vs. EM tree relative abundances, with two distinct frequency peaks at 100% AM and 100% EM dominance across the Eastern U.S (Figure 1a-b). Importantly, this signature persists even after controlling for environmental filtering by accounting for over 200 potential environmental drivers of tree species composition (Figure 1c). This was accomplished by fitting statistical models to forest mycorrhizal composition data, accounting for linear and non-linear community-environment relationships, and then adjusting relative abundance values to remove observed environmental signatures (see Methods). The bimodal distribution of forest types is consistent with the presence of alternative stable states fundamentally structuring the distribution of forest symbioses across North America, as well as previous analyses (16). By removing the signal of environmental filtering on the distribution of AM vs. EM forests, the remaining bimodal distribution (Figure 1c) demonstrates that this pattern cannot be explained solely by differing habitat preferences between AM and EM trees.

Commented [CA1]: I think this sentence is important, as it's what sets us apart from Lu and Hedin 2019, NEE. We account for 200+ environmental covariates, and allow them to have non-linear relationships, which is key, as it's easy to find nothing when you control for 5 things and throw straight lines at them. We were dinged for doing something similar to this in the last version.

We account for potential dispersal limitation by showing we account for residual spatial autocorrelation by using county (space) as a random effect. Without RSA, it's hard to argue we're seeing an artifact of dispersal limitation.

Fine w/ rewording or moving this sentence, we just really need to make these points

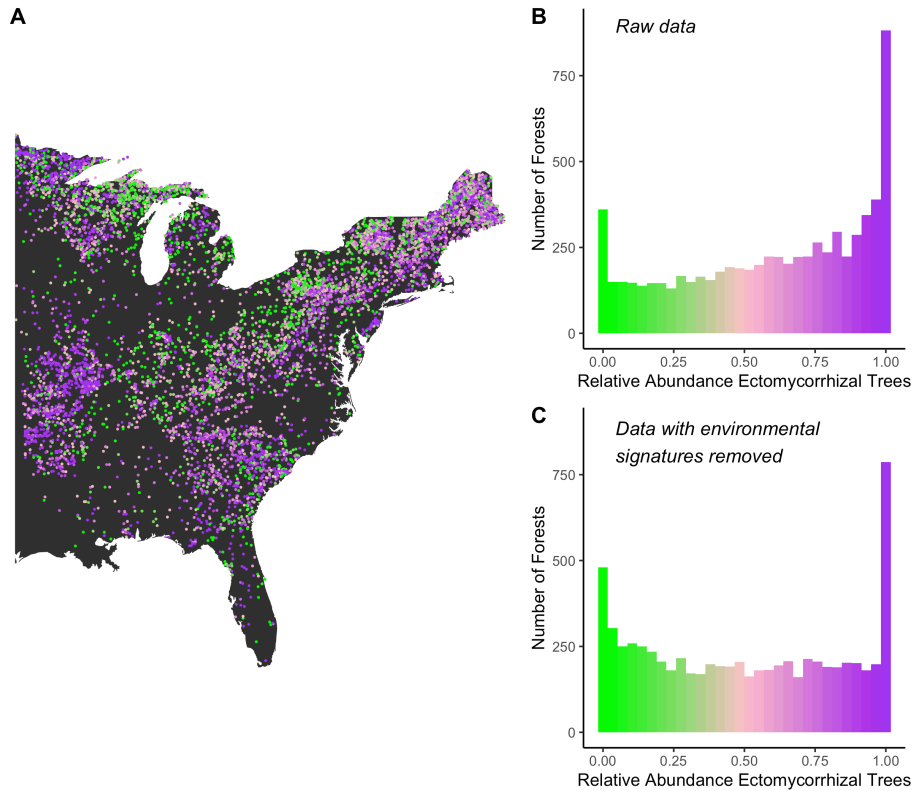


Figure 1. (A) the spatial distribution of sites used in this analysis. Colors represent the relative abundance of EM trees within a plot. (B) Histogram of the relative abundance of ectomycorrhizal trees by basal area across North America, controlling for effects of mean annual temperature, precipitation and nitrogen deposition. Values of 1 reflect forests with 100% ectomycorrhizal dominance and no arbuscular mycorrhizal trees, while values of 0 reflect forests with 100% arbuscular mycorrhizal dominance and no ectomycorrhizal trees. (C) The same data as presented in panel B, after removing environmental signals using statistical models.

Next, to examine the potential role of positive feedbacks in driving this observed bimodality within the forest mycorrhizal system over time, we analyzed tree recruitment and mortality across 6,965 repeated measure forest inventory plots. This revealed strong positive con-mycorrhizal feedbacks across the Eastern U.S.A. (Figure 2). Specifically, after controlling for tree size, environmental factors and stand conditions, an individual

EM tree is more likely to die in an AM forest than an individual AM tree, and AM trees are more likely to die in EM forests than EM trees (Figure 2A). Recruitment patterns were similar to mortality, with con-mycorrhizal relative abundance having a positive effect on con-mycorrhizal recruitment. Holding all else constant, EM tree recruitment is greater than AM recruitment in EM forests, and *vice versa* (Figure 2C). These findings were robust to spatially subsetting the data set across geographically defined ecoregions (Supplementary Figure 1). Furthermore, while we observed spatial autocorrelation in raw recruitment observations, consistent with dispersal limitation which could drive clumping individual species and therefore mycorrhizal types, final statistical models were able to capture and account for this autocorrelation, and therefore separate local community feedbacks from the potential influence of dispersal limitation (Supplementary Figure 2).

The observed patterns of recruitment and mortality were also apparent at the species level. Among the 22 most abundant AM and EM tree species, individual tree species are more likely to recruit and survive if their neighbors are of the same mycorrhizal type, and this pattern appears to be independent of hardwood vs. coniferous life history, and the basal area of conspecific trees (Figure 2C-D). Furthermore, individual AM trees also grew more slowly around EM neighbors, however no tree species grew faster in EM relative to AM conditions (Supplementary Figure 3). We suspect feedbacks in recruitment and mortality are stronger than growth, as mycorrhizal transfers of resources among trees are generally small, but small transfers may be critically important during seedling establishment and mortality inducing stress events (9). The observed patterns of recruitment and mortality potentially represent positive feedbacks that could generate and maintain bimodality of AM vs. EM forest states. However, while positive con-mycorrhizal recruitment and mortality feedbacks provide important demographic evidence consistent with alternative stable states of AM vs. EM forests, these patterns are also influenced by environmental drivers and demographic stochasticity. Strong evidence for positive con-mycorrhizal feedbacks would require that these patterns are strong enough to generate and maintain bimodality in the face of environmental heterogeneity, demographic stochasticity, and changes in forest structure over time.

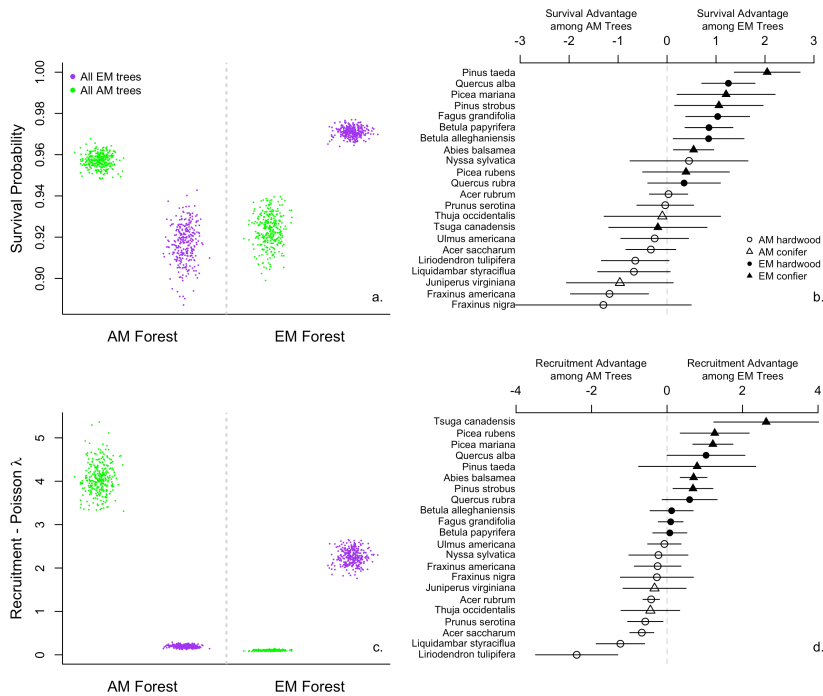


Figure 2. (A) survival probability of an individual arbuscular mycorrhizal (AM) tree (green) or ectomycorrhizal (EM) tree within a forest stand where all other trees are either arbuscular or ectomycorrhizal. Plotted values are estimates based on the full model, holding environmental conditions and stand structure constant, and drawn from the predictions 95% confidence interval. (B) Difference in Survival probability of individual tree species under EM relative to AM conditions. Differences are calculated on the logit-linear scale of the model. Positive values indicate an individual of a given tree species is more likely to survive within an EM dominated forest, and negative values indicate an individual is more likely to survive within an AM forest. Error bars represent the 95% confidence interval of the difference in means. (C) Recruitment rate of new arbuscular and ectomycorrhizal trees into a forest already dominated by arbuscular or ectomycorrhizal trees. Plotted values are estimates based on the full model, holding environmental conditions and stand structure constant, and drawn from the prediction 95% confidence interval. (D) Difference in recruitment rates of individual tree species under EM relative to AM conditions. Differences are calculated on the log-linear scale of the model. Positive values indicate an individual of a given tree species is more likely to recruit within an EM dominated forest, and negative values indicate an individual is more likely to recruit within an AM forest. Error bars represent the 95% confidence interval of the difference in means.

To test if positive con-mycorrhizal feedbacks in recruitment and mortality observed over a short time-scale are strong enough to generate and maintain alternative mycorrhizal stable states over longer time-scales, we ran a series of demographic simulations. Simulations were driven by empirically fitted statistical models of growth, recruitment and mortality. We initialized 1,000 forest plots with varying AM vs. EM composition and allowed them to grow for 150 years. We incorporated environmental heterogeneity by drawing plot-scale environmental conditions randomly from observed values across forests used to fit statistical models. We ran two sets of simulations. The first set was driven by empirical demographic models of growth, recruitment and mortality fit as a function of environment and stand characteristics, but without con-mycorrhizal predictors. The second set of simulations was driven by a second set of identical growth, recruitment and mortality models, except for the inclusion of an additional predictor for con-mycorrhizal relative abundance, capturing positive con-mycorrhizal feedbacks observed in the forest inventory data set. This second set of models were used to demonstrate positive con-mycorrhizal recruitment and survival feedbacks above (Figure 2a, 2c). In this way forest simulations are calibrated to, and driven by the data. We found simulations driven by demographic models fit without con-mycorrhizal predictors could not generate a bimodal distribution of AM vs. EM forests, while simulations driven by models fit with con-mycorrhizal predictors recovered the observed bimodal distribution of AM vs. EM forests (Figure 3). These findings suggest that observed con-mycorrhizal recruitment and mortality feedbacks are both necessary and sufficient in magnitude to generate and maintain alternative mycorrhizal stable states of the forest biome in the face of environmental heterogeneity, demographic stochasticity, and changes in forest structure over time.

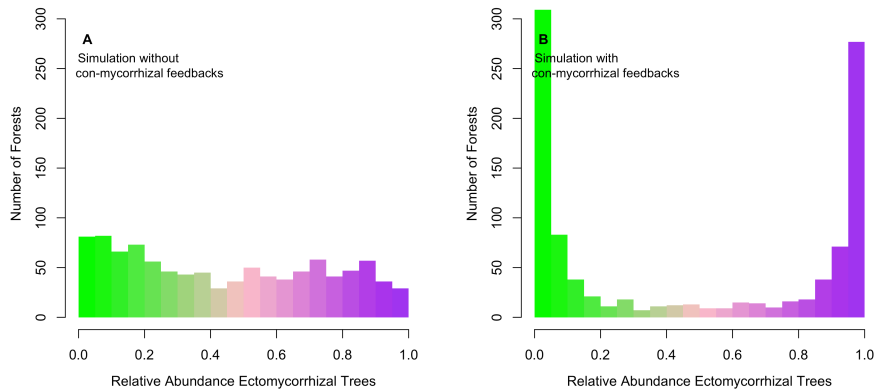


Figure 3. Histogram represents the relative abundance of EM trees within 1,000 simulated forests plots after 100 years. (A) Outcome of null demographic simulations, driven by growth, recruitment and mortality models fit without con-mycorrhizal density predictors. Very few plots are dominated by all AM or all EM trees. (B) Outcome of demographic simulations driven by growth, recruitment and mortality models fit with con-mycorrhizal density predictors. Plots dominated by AM or EM trees are the most common frequency categories.

The presence of alternative mycorrhizal stable states may fundamentally change temporal predictions of forest mycorrhizal composition over environmental gradients, and in turn, biogeochemical function. For instance, continental scale N deposition has been linked to a shift from EM to AM dominated forests (22). If this relationship is driven only by environmental filtering, then the relative abundance of AM vs. EM trees should be independent of the initial distribution of AM vs. EM forests across the landscape, given sufficient time (Figure 4A). However, once an EM or AM forest is established, it may resist transitions to the alternative state if con-mycorrhizal feedbacks are strong. If alternative stable states are present, then forest mycorrhizal transitions should exhibit hysteresis in response to N pollution (21), where the relative abundance of AM vs. EM trees depends on both the environment and the initial distribution of these forest types (Figure 4B). This hysteresis can take multiple forms – either a consistent shift, or community convergence at either one or both environmental extremes, but should exhibit a systematic sensitivity to initial distribution of AM vs. EM forests.

To understand the potential magnitude of hysteresis in forest mycorrhizal transitions under N pollution, we ran a second set of simulations, growing forests across a N deposition gradient, initializing 1,000 regenerating forests at the beginning of the simulation as either mostly EM dominated (80% of stands initialized with only EM trees, the remainder AM) or mostly AM dominated (80% of stands initialized with only AM trees, the remainder EM). Forest simulations were run for 400 years to minimize the influence of demographic lags on model outcomes. Remarkably, our simulations accurately reproduced the known relationship between a decline in EM forest dominance and N deposition (22). Furthermore, we observed substantial hysteresis across the N deposition gradient, where the relative abundance of EM trees across all forest plots strongly depended on the initial community state of the simulation (Figure 4C). For the same environmental conditions, the relative abundance of EM trees was much greater if the set of forest plots were initialized in an EM dominated state, than if they were initialized in an AM dominated state. Null simulations show the magnitude of observed hysteresis cannot be explained in the absence of mycorrhizal feedbacks (Supplementary Figure 4). The mechanisms behind this resistance may be linked to common mycorrhizal networks, N cycle feedbacks, and more. Given the central role of mycorrhizal type in influencing forest functioning, this hysteresis has implications for ecosystem-level responses to global change, and our adaptive management efforts to reverse the impacts of N deposition and other global change drivers at broad spatial scales.

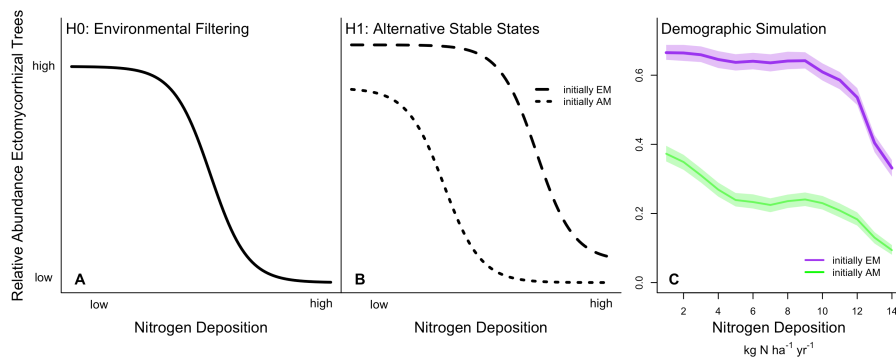


Figure 4. (A) Environmental filtering predicts the relative abundance of AM vs. EM trees across a landscape as a function of nitrogen deposition should be driven by

environmental filtering, and independent of the initial distribution of AM vs. EM forests. (B) If alternative stable states are present then the relative abundance of AM vs. EM trees should exhibit hysteresis in response to N deposition. The relative abundance depends not only on environmental conditions but also the initial community state of the forest (initially AM or EM dominated). (C) Results of two sets of demographic simulations where 80% regenerating forests started in an EM dominated state (purple line) or an AM dominated state (green line). Shaded regions represent the 95% bootstrap confidence interval of the mean relative abundance of EM trees across each set of forest simulations.

Researchers have long suspected contrasting forest mycorrhizal strategies may generate feedbacks which reinforce their establishment and persistence (15). However, only recently has sufficient knowledge accrued to understand forest mycorrhizal composition and turnover at massive spatial scale, allowing us to finally empirically test this hypothesis. Our findings are also consistent with foundational work on alternative ecosystem stable states of eastern hemlock and sugar maple forests in northern Michigan, U.S.A. (23, 24). Yet these two species also represent contrasting mycorrhizal types, with sugar maple forming AM and eastern hemlock forming EM symbioses. We suggest these findings are likely to be a specific example of a more general and widespread phenomenon. Given the prominent role of mycorrhizal fungi in shaping the biogeochemistry of their ecosystems, mycorrhizal feedbacks likely extend to other tree species and much larger spatial scales. In addition, previous work on common mycorrhizal networks has shown positive associations between con-mycorrhizal, but not hetero-mycorrhizal tree species (8, 10, 11). We emphasize that both N-cycle feedbacks as well as common mycorrhizal networks within mycorrhizal types may act in concert to mediate positive feedbacks that generate and maintain alternative mycorrhizal stable states.

It is important to recognize that the presence of con-mycorrhizal feedbacks does not negate the role of environmental filtering in structuring the distribution of AM vs. EM forest symbioses at broad spatial scales (1, 22). However, within a continental scale envelope of environmental conditions, prediction of AM vs. EM forests can only be made probabilistically, due to the presence of strong positive con-mycorrhizal feedbacks, which give rise to priority effects and alternative stable states (4). This is analogous to how high vs. low rainfall is linked to the presence of savannah vs. forest, but at intermediate rainfall alternative stable states of forest vs. savannah emerge due to positive feedbacks linked

to fire (25). These findings may provide mechanism behind sharp transitions in the spatial distribution of AM vs. EM ecosystem types observed at global scale (1). Finally, this analysis has lumped incredible taxonomic and functional diversity of both plants and fungi into AM vs. EM functional groups. We recognize different tree species and different mycorrhizal fungi have diverse and complex ecological strategies (15), and these strategies may modify the strength of con-mycorrhizal density dependence and its environmental sensitivity (Figure 2). We see exploring this diversity as the next frontier in understanding how the predominant form of tree mycorrhizal symbiosis shapes the biogeography and function of the forest macrosystem.

Alternative stable states of terrestrial ecosystems have long been hypothesized but rarely demonstrated, in part because the empirical evidence needed is notoriously difficult to obtain at sufficient spatial and temporal scales (21, 25). This is particularly true for belowground microbial systems where it is exceptionally difficult to isolate the drivers of community change. In addition, evidence to support such broad-scale feedbacks can only be correlative, rather than causative, so multiple lines of evidence are needed. Here we present multiple lines of evidence, each relying on independent analytical approaches, to provide strong support for the presence of alternative mycorrhizal stable states within the temperate forests of eastern North America. These alternative stable states likely arise from a combination of divergent plant traits (26), interactions between mycorrhizal fungi and free-living soil microbial communities at the molecular scale (3, 27), and common mycorrhizal networks (8, 10, 11). This combination of plant and microbial feedbacks then ripples through the ecosystem to affect the patterning of forest mycorrhizal symbioses across the continent. By highlighting how the soil microbiome shapes the functioning and responsiveness of entire forests, this work demonstrates the emerging importance of the forest microbiome for understanding the biogeography and function of the forest macrosystem. Given the striking differences in carbon cycling and storage between AM and EM-dominated systems (5, 6), the presence of alternative mycorrhizal stable states requires a rethinking of how forest composition and function vary across time and space, as well as the implications of these predictions for the global carbon cycle and in turn potential future states of the Earth.

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Methods:

Data collection for the United States (US) Forest Service Forest Inventory Analysis Database: All data used in this set of analyses comes from the US Forest Service's Forest Inventory and Analysis database, version 7 (28). We further subsetted to sites within the Eastern US where re-measurement intervals are standardized, which facilitated fitting of demographic models and demographic simulations, as done in previous analyses (29) (Figure 1). We only considered sites where all subplots were in a forested condition and excluded plantations as well as any sites with evidence of active management or harvesting. Trees were assigned AM or EM association as in previous analyses (22). Once mycorrhizal associations were assigned, we further subsetted the data set to sites where >90% of basal area was associated with either AM or EM associated trees. Therefore, a plot with 50% EM basal area and 50% AM basal area would be included in this analysis, however any plot with >10% mixed mycorrhizal, arbutoid, ericoid or non-mycorrhizal basal area would be excluded. This filter excluded 15% of all forested sites. Further investigation showed 2/3 of excluded sites (i.e. 10 of the 15%) were due to a high abundance of *Populus*, a well-known dual AM-EM tree genus. The remainder of exclusions were primarily driven by trees where mycorrhizal strategy was unknown. Our final dataset included 6,965 unique forest sites, re-censused at a ~5-year interval, with complete environmental covariates, comprised of 200,363 trees.

Environmental covariates: To control for potential environmental filtering we extracted information on 204 environmental variables that could be related to forest and mycorrhizal ecology. These environmental variables captured variation in climatic, soil nutrient, soil chemical, soil physical and vegetative indices, radiation and topographic variables and anthropogenic influence (Supplementary Data File 1). We explicitly did not include the soil chemical factors %C, %N, C:N or soil pH, as these are all hypothesized mechanisms by which tree species modify the soil environment to generate alternative stable states. All covariate map layers were resampled and re-projected to a unified pixel grid in EPSG:4326 (WGS84) at 30 arcsec resolution (approximately 1 km² at the equator). Layers with a higher original pixel resolution were down sampled using a mean aggregation method; layers with a lower original resolution were resampled using simple

up sampling (that is, without interpolation) to align with the higher resolution grid (30). To reduce predictor dimensionality we performed principle component analysis on all environmental predictors, and included the first ten principle components which captured ~83% of the variation in the original 204 environmental drivers. These ten environmental predictors were included in all statistical models of tree growth, recruitment and survival, as well as the relative abundance of EM trees within a plot.

In addition to the above environmental variables, we also included a metric of historic N deposition loading. By including this in addition to the above ten environmental principle components, we could ask questions about the direct effect of N deposition, independent of these other environmental factors. 15 year mean annual wet and dry N deposition was assigned at 0.25° resolution (31). We summed wet and dry N deposition to estimate total N deposition. Previous work has shown 15 and 30-year wet N deposition data are strongly correlated, and are representative of historical patterns of N deposition loading across the United States (22).

The FIA does not report site coordinates with exact precision, and this affects the accuracy of our environmental drivers. Plot locations are limited in accuracy to ~800m, and then 20% of observations are randomly swapped at the county level. Because variation in environmental factors are much larger across counties than within, this limited spatial precision will increase parameter uncertainty associated with these environmental drivers, but does not prevent us from detecting large-scale patterns, the goal of this analysis (22).

Testing for bimodality in the contemporary distribution of AM and EM forests: Alternative stable state theory predicts that, within some envelope of environmental conditions, ecosystem types should exhibit a bimodal distribution (4, 21). However, if exogenous environmental conditions that also affect the abundance of AM vs. EM trees within a forest are also bimodal or affect the distribution of trees in non-linear ways, this may generate bimodality in the distribution of AM vs. EM forests. To control for this potential confounding, we fit generalized additive models to EM relative abundance to determine the effect of environmental drivers on the distribution of EM vs. AM relative abundance. In order to use a normal error distribution, values of the relative abundance of EM trees

were transformed from the interval [0-1] to (0-1) (32), then logit transformed to meet regression assumptions (analogous to beta regression). These values were modeled as a function of the ten environmental principle components described above, and nitrogen deposition. County was included as a random effect to account for potential residual spatial autocorrelation. All predictors, except for county level random effects, were fit as independent spline terms with a maximum number of potential “knots” set to 5 to account for potential non-linearities, while avoiding overfitting. Splines were fit using a penalized thin-plate spline regression method (33). Once the model was fit, we removed environmental signatures from transformed relative abundance values by subtracting fitted model values from observed values, and then adding the mean value of each environmental predictor transformed by its associated spline function. These values were then re-projected onto the original (0,1) scale via an anti-logit transformation. We tested for bimodality of both the raw and environmentally-detrended distributions using a two-tailed Hartigan's Dip Test for statistical unimodality (34).

Testing for demographic neighborhood effects that would reinforce AM vs. EM dominated states: Changes in the overall relative abundance of AM vs. EM trees in a forest are fundamentally driven by differences in AM vs. EM forest demographic processes of growth, recruitment and mortality (or survival). We modeled each of these processes as a function of environmental factors, individual tree and stand characteristics (tree size, stand stem density and stand basal area), and the relative abundance of EM trees in the forest (our con-mycorrhizal predictor), to quantify the influence of con-mycorrhizal density dependence. Once models were fit, we held environmental, individual and stand characteristics constant and used the model to understand the sign and magnitude of con-mycorrhizal neighborhood effects. This allowed us to answer questions like, is an AM tree less likely to die in an AM forest than an EM tree? Or, does an EM tree grow more in an EM forest relative to an AM tree?

Modeling tree growth: Tree growth was modeled at the individual tree level across the most recent 5-year time interval for each forest site using a generalized additive model (gam) within the mgcv package (33) for R statistical software (35). We fit separate models for AM and EM associated trees. Within each model, current tree diameter was modeled

as a function of an individual tree's previous diameter, total plot basal area, stem density within a plot, all environmental covariates, and the relative abundance of EM trees within a plot (i.e. the con-mycorrhizal predictor). County was included as a random effect to account for potential residual spatial autocorrelation. All predictors, except for county level random effects, were fit as independent spline terms with a maximum number of potential "knots" set to 5 to account for potential non-linearities, while avoiding over-fitting, using a penalized thin-plate spline regression method (33).

Modeling tree recruitment: Recruitment of AM and EM trees were modeled separately at the plot level across the most recent 5-year time interval for each forest site within the GAM framework using a Poisson distribution implemented within the mgcv package (33) for R statistical software (35). Recruits are defined as all individuals in the current census greater than 12.7cm diameter which were not present in the previous census. Recruitment of AM or EM trees was modeled as a function of plot basal area, stem density, basal area of con-mycorrhizal trees, all environmental covariates, and the relative abundance of EM trees within a plot (i.e. the con-mycorrhizal predictor). The basal area of con-mycorrhizal trees within the plot was included as a term in addition to the con-mycorrhizal predictor to account for the fact that recruitment generally increases with the abundance of a focal species or functional group within a given site, i.e., the apple does not fall far from the tree (36). County was included as a random effect to account for potential residual spatial autocorrelation. All predictors, except for county level random effects, were fit as independent spline terms with a maximum number of potential "knots" set to 5 to account for potential non-linearities, while avoiding over-fitting, using a penalized thin-plate spline regression method (33).

Modeling tree mortality (survival): Tree mortality probability was modeled at the individual level across the most recent 5-year census interval within the GAM framework using a binomial distribution implemented within the mgcv package (33) for R statistical software (35). We fit separate models for AM and EM associated trees. Within each model, tree mortality probability was modeled as a function of an individual tree's previous diameter, total plot basal area, stem density within a plot, all environmental covariates, and the relative abundance of EM trees within a plot (i.e. the con-mycorrhizal predictor). All predictors were fit as independent spline terms with a maximum number of potential

“knots” set to 5 to account for potential non-linearities, while avoiding over-fitting, using a penalized thin-plate spline regression method (33). County was included as a random effect to account for potential residual spatial autocorrelation. Results are visualized as survival probability (1 – mortality probability).

Species level growth, recruitment and mortality models: We fit growth, recruitment and mortality models as above, but at the species level for the 11 most abundant AM and 11 most abundant EM tree species, based on the number of individual stems within the dataset. Model structures and covariates were identical, save for recruitment, where con-mycorrhizal basal area was replaced with the basal area of the particular species being modeled within a plot (i.e. conspecific basal area).

Ecoregion level growth, recruitment and mortality models: The study region considered here contains multiple ecological sub-regions with distinct combinations of tree species and potentially unique interactions between AM and EM associated trees at the functional group level. To understand if our findings across the Eastern U.S. generalized to ecological sub-regions, we refit growth, recruitment and mortality probability models as above to five regional subsets of the dataset. Regional subsets were chosen using EPA level 2 ecoregions that contained at least 1,000 unique forest inventory plots (37).

Demographic Simulation 1: Testing if demographic neighborhood effects are sufficient to maintain alternative stable states: Positive con-mycorrhizal effects in and of themselves are not sufficient to generate and maintain alternative stable states. Positive con-mycorrhizal effects must be sufficient in magnitude to maintain alternative stables within a dynamic forest, in the face of environmental heterogeneity, demographic stochasticity and changes in stand structure through time. To test if observed con-mycorrhizal effects were sufficient in magnitude to maintain alternative mycorrhizal stable states, we ran a series of demographic simulations driven by empirically fitted demographic models of growth, recruitment and mortality. Simulations are initialized with 1,000 forest plots comprised of 20 trees at 12.7cm DBH (the smallest tree measured in FIA surveys) within a plot area identical to that of the FIA, and then run for 100 years. We incorporated stand

replacing disturbance at a probability of 0.36% per year. This number was derived based on the overall North American stand replacing disturbance probability of 0.9% a year, and then reducing this number by 60% to exclude stand replacement associated with human harvest (38). When stands were cleared, stem density was reset to 20 trees per plot. Regenerating trees were assigned EM vs. AM status using the stands prior relative abundance of EM trees as the probability each new regenerating tree being EM vs. AM. We incorporated environmental heterogeneity by assigning each plot a set of environmental covariates drawn randomly from the combinations of environmental covariates observed across the forest plots used to fit demographic models. We first asked if empirically fitted demographic models are sufficient to generate bimodality of AM vs. EM dominated forests after 100 years. We then ran a second simulation, driven by a second set of "null" demographic models which were fit without con-mycorrhizal predictors (the relative abundance EM trees within a plot) to ask if demographic lags or other aspects of our demographic models or simulation could generate bimodality of AM vs. EM forests without any feedback mechanism. Half of all forest plots in each simulation were initialized with all AM trees, and the other half with all EM trees. This gave our null simulation the best chance to recapitulate bimodality of AM vs. EM forests due to demographic lags and therefore disprove our hypothesis.

Demographic Simulation 2: Testing for hysteresis in AM vs. EM forest dominance across gradients of N pollution: Hysteresis in community transitions across environmental gradients is a hallmark of alternative stable states (21). We ran two sets of demographic simulations described above, across a gradient of N deposition rates from 1 to 14 kg N ha⁻¹ yr⁻¹. In the first set of simulations 800 of 1,000 forest plots were initialized at 100% EM dominance (the remainder initialized as AM), and in the second set of simulations all 800 of 1,000 forest plots were initialized at 100% AM dominance (the remainder initialized as EM). Simulations were run forward for 400 years to minimize the influence of demographic lags on simulation outcomes. We visualize the relative abundance of EM trees across all plots at the end of the simulation as a function of N deposition.

Data and Code Availability: All forest data used in this analysis are available from the FIA data mart (<https://apps.fs.usda.gov/fia/datamart/>). All code used to work up raw data to analysis ready products, analyze data and generate figures can be found at https://github.com/colinaverill/altSS_forest_mycorrhizas. All environmental covariate data sources are publicly available, and detailed in Supplementary Data File 1.

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