**Competition leads to phylogenetic divergence in plant community of an unmanaged lawn recovering from herbicide spray**

**Introduction**

Herbicides shape plant community dynamics by differentially affecting species' survival, growth, and recovery, thereby altering their taxonomic and functional composition (Grundy et al., 2011). Some species demonstrate resilience and recover rapidly after herbicide exposure, while others fail to re-establish (Iriart et al., 2021; Powles & Yu, 2010; Gratani, 2014; Burns & Strauss, 2011). Environmental conditions, resource availability, and species-specific phenotypic traits may influence these differential responses (Helmus et al., 2010; Iriart et al., 2021; Délye et al., 2013; Gratani, 2014; Kumordzi, 2015; Fritschie et al., 2014; Violle et al., 2011). However, the extent to which evolutionary history shapes these responses amidst other competing factors is still debated.

Phylogenetic conservatism, the tendency of closely related species to share similar ecological traits and niches due to their shared evolutionary history, is important to understanding species assembly and diversity patterns. Closely related plant species often share phenotypic traits, which could result in similar levels of resilience or susceptibility to disturbance (Helmus et al., 2010; Losos, 2008; Cavender-Bares & Reich, 2012; Violle et al., 2011; Schoener, 2011). However, there is evidence to the contrary: Close relatives might not respond similarly to anthropic activities if competition between similar species takes a toll on the locally coexisting species (Violle et al., 2011; Fritschie et al., 2014; Naughton et al., 2015). This is largely due to Darwin’s (1859) suggestion: species sharing close evolutionary relationships are more similar and compete more intensely with one another than with more distant species. This principle is referred to as the competition-relatedness hypothesis and is supported by the theory of limiting similarity (MacArthur & Levins 1967; Abrams, 1983), which emphasizes that species must differ in their ecological niches to coexist locally. If species are too alike, the one with a competitive disadvantage will be driven towards depletion (or a worst case: extinction) due to competitive exclusion (Hardin, 1960; Jaeger, 1974; Johnson & Bronstein, 2019; Levin et al., 2020). As a result, competition among closely related species may counteract phylogenetic conservatism. If related plant species experience stronger competition with one another than with more distantly related species, herbicide resilience within a closely knit clade could lead to intense intra-clade competition, ultimately favouring the persistence of more distantly related species. Thus, competition may drive divergence in herbicide resilience, potentially counteracting phylogenetic conservatism in some cases.

Despite extensive research on plant-herbicide interactions, the role of phylogenetic relatedness in shaping plant species’ responses to disturbances from herbicide application remains understudied. Understanding whether plant responses to herbicide application—survival and/or recovery—are phylogenetically conserved (or not) can provide valuable insights into community assembly and species coexistence in disturbed environments (Violle et al., 2011; Germain et al., 2016; Zepeda & Martorell, 2021). Identifying the balance between evolutionary constraints and competitive interactions in shaping plant community responses can provide valuable insights for ecological management and conservation in both natural and human-influenced landscapes (Grundy et al., 2011; Pellissier et al., 2014; Lemos‐Costa et al., 2024).

This study aims to assess the phylogenetic conservatism of plant responses to herbicide application in an unmanaged lawn. Specifically, I will determine whether closely related species exhibit similar survival and recovery patterns, as reflected in changes in their percentage ground cover over time. For this study, ground cover would be regarded as the living (green) plants as seen from a vertical aerial observation. In a dense grass community, the (percentage) ground cover is a good way to learn how much competitive space a plant species has (Anderson, 1986; Damgaard, 2011). In limited space, ground coverage is a zero-sum game; plants that take higher cover have more access to sunlight and also consequentially deprive other plants of space and sunlight. If phylogenetic conservatism influences herbicide resilience, I expect closely related plant species to exhibit similar responses immediately following herbicide application, leading to increased phylogenetic clustering among surviving species. However, as ground cover increases during post-spray (recovery), competitive interactions may disrupt this pattern, leading to greater phylogenetic divergence over time. If competition structures communities, then the co-existing plants should be less related than expected by chance. By examining how phylogenetic relatedness influences plant survival and recovery in response to herbicide disturbance, this study will contribute to a broader understanding of species persistence in dynamic environments.

**Materials and Methods**

**Study Site**

The study was conducted from February to April 2023 on an enclosed, unmanaged lawn behind Egborge Museum at the University of Benin, Benin City, Nigeria (longitude: 6.392° N, latitude: 5.613° E). The university is located in southern Nigeria, within a tropical rainforest environment. As shown in Figure 1, the site measures 23.77 m by 11.58 m. During the survey period, mean ambient hourly temperatures ranged from 25 to 34 °C (Accuweather, 2023).

**Experimental Design and Herbicide Application**

To allow for independent replication and to observe potential spatial autocorrelation, the study site was divided into six plots (A-F; Figure 1). Each plot contained six subplots, but data were aggregated at the plot level to reduce random noise. A subplot is a 1 × 1 m area measured by an equally sized quadrant.

Before herbicide application, plant cover was surveyed as a baseline (week 0). The entire study site was then treated with a non-selective systemic herbicide, Forceup™, which contains glyphosate as the active ingredient. The herbicide was applied at the recommended rate of 6 L/ha using a manually pressurized, two-litre handheld sprayer, with a solution concentration of 48 mL of glyphosate per litre of water.

**Data Collection**

Plant cover data were collected over approximately 11 weeks, specifically at weeks 0 (pre-spray), 3, 6, 9, and 11. Cover was estimated on a scale of 0–100%, where 0% indicated that the plant was not visible from a vertical aerial perspective, and 100% meant that a plant species completely dominated the 1 × 1 m quadrat, with no visible ground. Dead or fallen leaves were excluded from the cover estimation. The survey concluded at week 11, as continued measurements became impractical due to some plant species reaching heights of up to 0.5 m, making ground cover estimation unreliable.

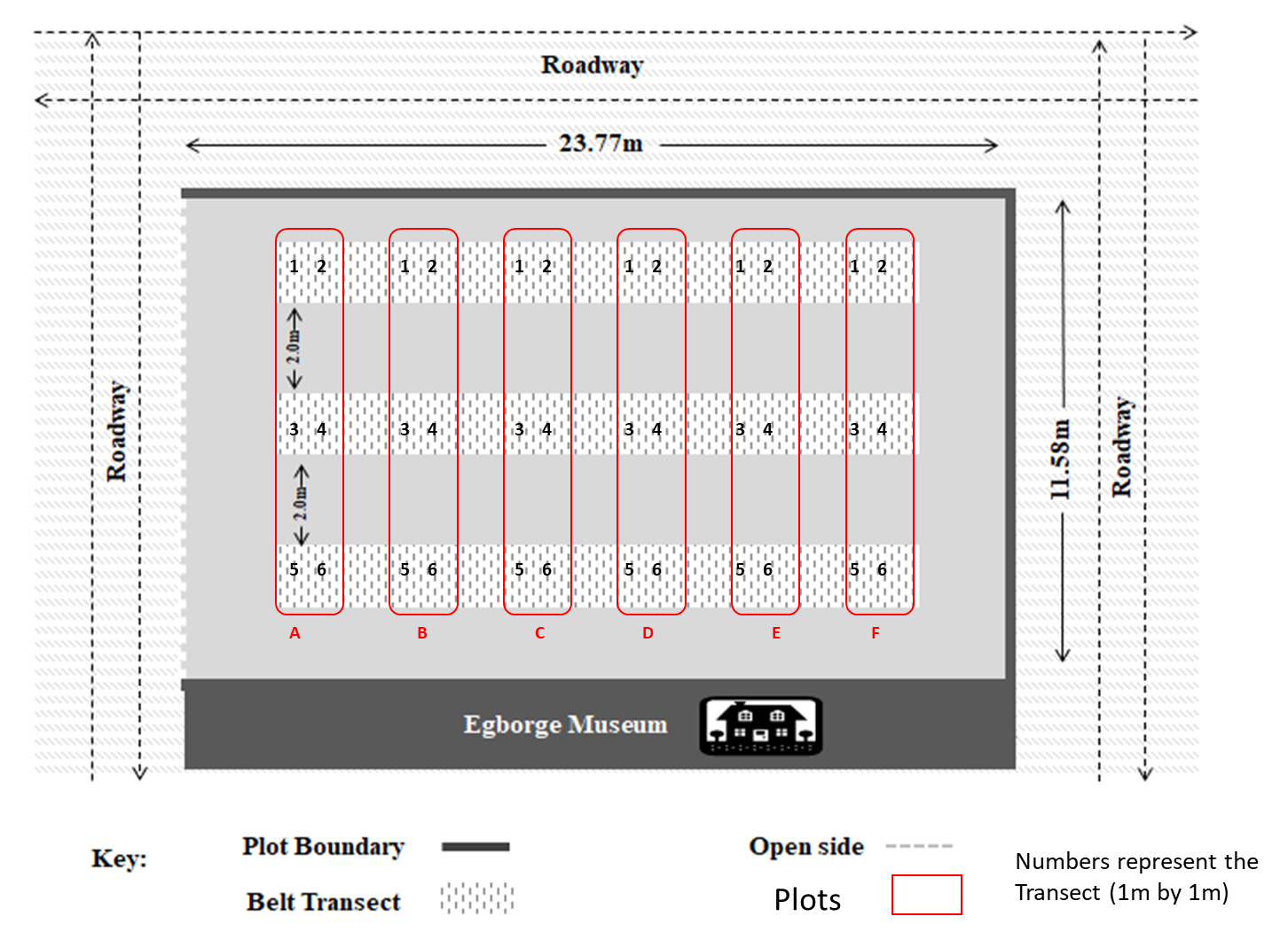


Figure 1: Experimental plot, adapted from Osawe N.E.'s undergraduate thesis (2023)

**Phylogenetic and statistical analysis**

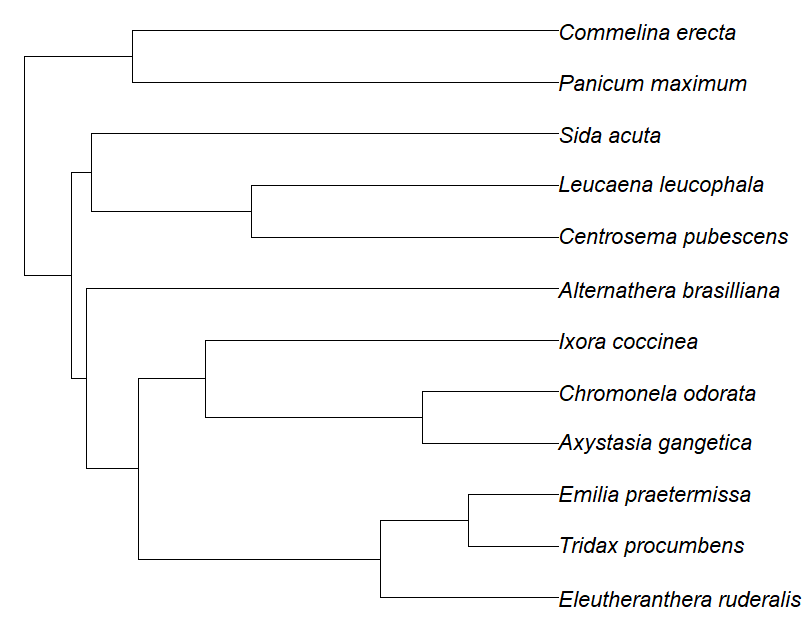
Phylogenetic trees of the plants were assembled using the “V.PhyloMaker” R package. This package matches the taxonomic names of our study plant species to a backbone phylogeny derived from the Global Backbone of Tree for Biodiversity (GBOTB) with extended coverage (Jin & Qian, 2019). This approach allows for the inclusion of species that may not be present in the reference phylogeny by placing them within their most likely evolutionary position.

Phylogenetic distances were computed and matched with longitudinal percentage ground cover data. We tested for significant phylogenetic signal, relative to a random distribution of the traits observed, given the topology and branch lengths of the ML phylogeny, with 999 replicates. To estimate phylogenetic conservatism (or divergence), we will use Blomberg's K statistic. Blomberg’s K is used as a metric of phylogenetic signal, where K is calculated as the ratio of the observed phylogenetically correct mean-square error divided by the mean-square error of the data, standardized by the expectation under Brownian motion (Blomberg et al. 2003). K = 0 indicates no phylogenetic signal, 0 < K < 1 indicates that closely related species resemble each other less than expected under the BM model of trait evolution, K = 1 indicates phylogenetic signal as expected by BM evolution, and K > 1 indicates high phylogenetic signal, with closely related species resembling each other more than expected under BM (Blomberg et al., 2003).

Welch’s t-tests would be used to assess significant differences in the effect of spraying on [1] Blomberg's K values and [2] the plant ground cover (%) before and after spraying. Furthermore, an orthogonal polynomial mixed-effects model would be used to examine the relationship between the plant’s ground cover (%) and the strength of the phylogenetic signal (measured using Blomberg’s K statistics). Plots were included as a random effect to control for variation across experimental units. Analyses were conducted in R (version 4.4.0.1). All data set and R script used in the analysis are present in this GitHub repository:

**RESULTS**

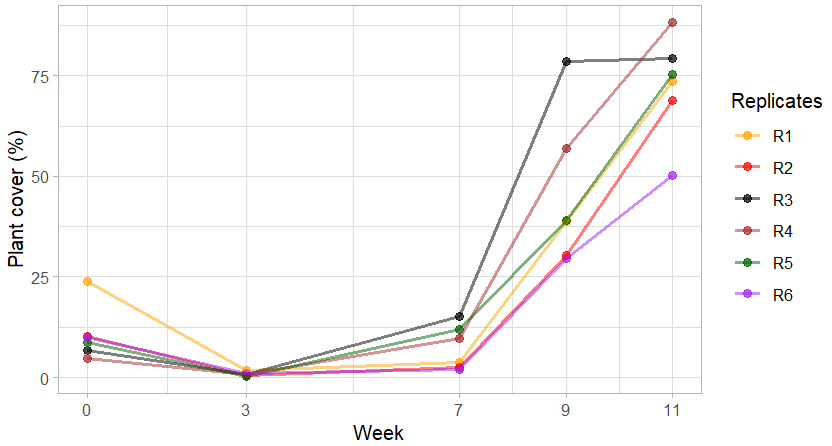
All plant species from our study belong to 12 distinct species, and their evolutionary tree is shown in Figure 1. The plant species include: *Chromolaena odorata* (Rutaceae), *Axystasia gangetica* (Acanthaceae), *Ixora coccinea* (Rubiaceae), *Sida acuta* (Malvaceae), *Alternathera brasiliensis* (Amaranthaceae), *Panicum maximum* (Poaceae), *Emilia praetermissa* (Asteraceae), *Tridax procumbens* (Asteraceae), *Commelina erecta* (Commelinaceae), *Centrosema pubescens* (Fabaceae), *Leucaena leucophala* (Fabaceae), and *Eleutheranthera ruderalis* (Asteraceae).



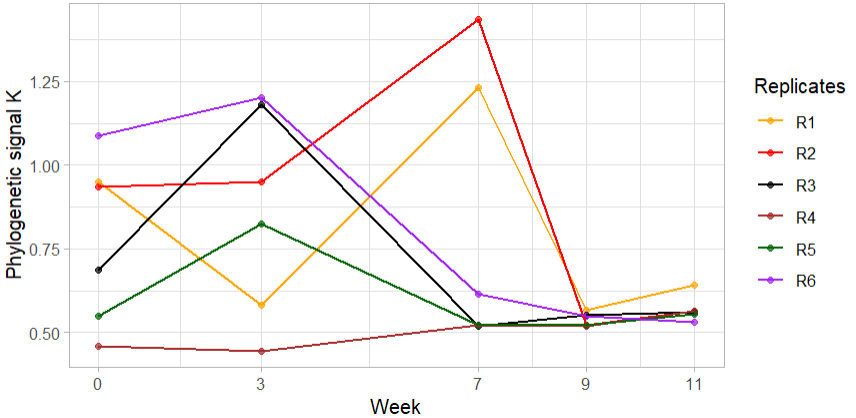
**Figure 1**: Phylogenetic tree showing the evolutionary relationships between the plants in the study.

Before herbicide spray (week 0), the plant cover was at 10.75±2.77% but reduced to 0.84±0.22% after the spray (in week 3). However, the plant responded positively with time, increasing rapidly up to 72.6±5.20% at the end of the survey (week 11), as shown in Figure 2.

The spray of herbicide significantly (t= 3.54, p<0.05) impacted the ground cover (%) of plants, as seen in the reduction in cover between pre-spray and post-spray (week 3). However, this was not so with the phylogenetic signal, which did not change significantly (t= -0.52, p>0.1) between the pre-spray and the immediate post-spray period (week 3). This is so despite the high reduction (-92.1%) in mean percentage of plant cover following the spray (Figure 2), indicating that the spray of herbicide had no significant implication on the plant community’s apparent phylogenetic structure, despite the high reduction (-92.1%) in plant cover following the spray (Figure 2). Over time, lower phylogenetic signals were observed towards the last few weeks of the survey (Figure 3)—the same period where high plant cover (%) was observed.

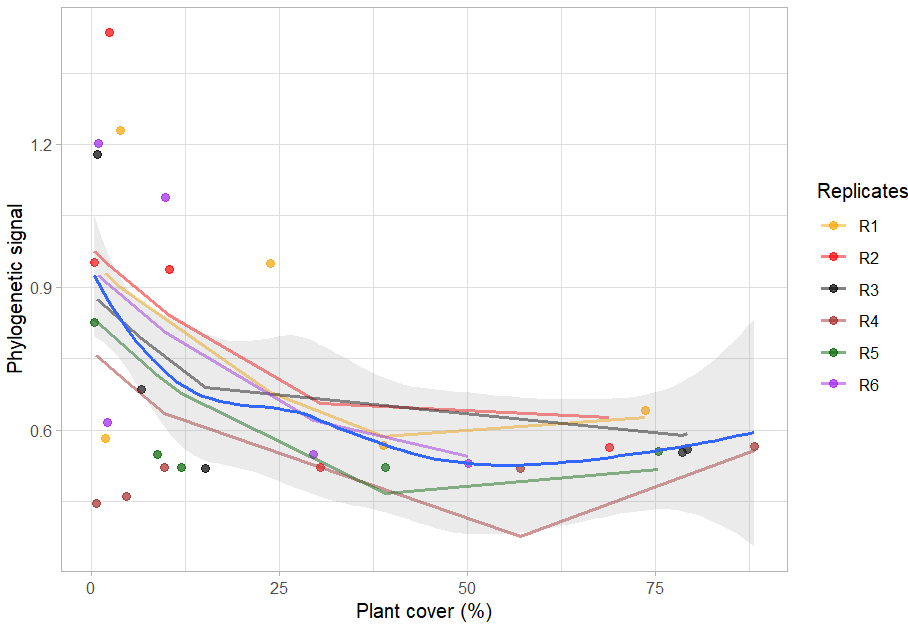


**Figure 2**: Percentage ground cover of plants on each plot at every survey week. Week 0 is a pre-spray, while weeks 3-11 are post-sprays.



**Figure 3**: Phylogenetic signal (Bloomberg K values) of plants on each plot at every survey week. Week 0 is a pre-spray period, while weeks 3-11 are post-spray periods.

An orthogonal polynomial mixed-effects model was used to assess the relationship between phylogenetic signal and plant ground cover, incorporating a quadratic term to account for potential nonlinear effects. The linear term for ground cover was significantly negative (β = -0.596, SE = 0.225, p = 0.015), suggesting that the phylogenetic signal decreased as plant ground cover increased. However, the quadratic term was marginally significant (β = 0.461, SE = 0.230, p = 0.057), indicating a possible nonlinear relationship, where the phylogenetic signal may initially decline but later stabilize or slightly increase at higher ground cover levels (Figure 4).



**Figure 4**: Relationship between (strength of) phylogenetic signal and plant cover (%). Each line indicates a different replicate.

**DISCUSSION**

This study has shown that herbicide spray does not significantly affect the phylogenetic signal. 12 distinct species of plants were observed in this study, which is high plant species richness for a lawn. The nearly closed site in this study makes the work interesting. The landscape makes the populations closed and slows the prospects of immigrating species.

Unlike many other studies, there was no phylogenetic signal that did not change as a result of herbicide application. This was not what I envisioned, as herbicides have been reported to selectively kill plants based on phylogenetic similarity.

Rapid increase in the plant cover would likely be a result of the rain pours between late March and April. These months mark the beginning of the wet season in the southern part of Nigeria. The downpour of rain caused a high rate of plant recovery from the herbicide spray but also led to competition for space and sunlight. Perhaps, broad-leaved plants would dominate in this study survey, and plants that trail on the ground. As compared to those plants that rather grow upwards

A key finding of this study is the significant increase in plant cover over time, accompanied by a decline in phylogenetic signal during the later weeks of recovery. This pattern supports the competition-relatedness hypothesis (Darwin, 1859; Violle et al., 2011), which posits that closely related species compete more intensely due to their ecological similarity. As plant cover increased, competition for space and light intensified, likely favoring species with greater competitive ability rather than those that simply survived the herbicide. The observed phylogenetic divergence suggests that competition, rather than herbicide tolerance, was the primary driver of community assembly in the latter stages of the study.

Menalled et al. (2023) demonstrated the use of the competition exclusion principle to manage weed, by experimenting with the use of weed suppression services from cover crops can be an important tool to reduce reliance on herbicides and tillage for weed management.

Boutin et al. (2019) described the effect of completion saying that competitive interactions triggered significant changes in the response of plant species to sub-lethal doses of glyphosate and metsulfuron-methyl herbicides, underpinning the need for factoring species interactions such as competition into ecological risk assessments of herbicides.

Levin had shown that phylogenetically distinct species compete less with their local communities, but the relationship disappears if the resident community is defined at a larger spatial grain. Levin also suggested that incorporating functional traits in addition to phylogenetic relationships would help predict the response of plants to competition.

**Limitations and Future Studies**

I am aware that species turnover can significantly affect the result of our study. While no significant change was observed in the phylogenetic signal before and 3 weeks after spray, it is possible that there would be high species turnover that set the tone for competition and rapid growth of plants in the lawn during the 7-11th week of the survey.

Since the study is not a controlled experimental one, it is difficult to know if some phenotypic trait expressions such as trailing, broad leaves, pattern of growth and phototropism is affecting the competitive advantage of plants, over phenotypic resemblance.

Since this study supports Dawrin's idea of the competitive exclusion principle, likely, the plants that are surfacing (for light and space) have different niche requirements. Those at the surface would deprive others of sunlight

**CONCLUSION**

This study supports Darwin’s competitive exclusion principle, demonstrating that competition among closely related species can lead to phylogenetic divergence in a recovering plant community. While herbicide application significantly reduced plant cover, it did not alter phylogenetic signal immediately post-spray. Instead, as plants recovered, competitive interactions favored the persistence of more distantly related species, leading to a decline in phylogenetic signal over time. These findings highlight the importance of competition in shaping plant community assembly after disturbance, emphasizing the need for integrated approaches that consider both evolutionary history and functional traits in ecological management and conservation.

**REFERENCES**

Jin, Y., & Qian, H. (2019). V. PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography*, *42*(8), 1353-1359.

Lovette, I. J., & Hochachka, W. M. (2006). Simultaneous effects of phylogenetic niche conservatism and competition on avian community structure. *Ecology*, *87*(sp7), S14-S28.

Damgaard, C. (2011). Measuring competition in plant communities where it is difficult to distinguish individual plants. *Computational Ecology and Software*, *1*(3), 125-137.

Anderson, E. W. (1986). A guide for estimating cover.

Powles, S. B., & Yu, Q. (2010). Evolution in action: plants resistant to herbicides. *Annual review of plant biology*, *61*(1), 317-347.

Iriart, V., Baucom, R. S., & Ashman, T. L. (2021). Herbicides as anthropogenic drivers of eco‐evo feedbacks in plant communities at the agro‐ecological interface. Molecular Ecology, 30(21), 5406-5421.

Délye, C., Jasieniuk, M., & Le Corre, V. (2013). Deciphering the evolution of herbicide resistance in weeds. Trends in Genetics, 29(11), 649-658.

Kumordzi, B. B., Wardle, D. A., & Freschet, G. T. (2015). Plant assemblages do not respond homogenously to local variation in environmental conditions: functional responses differ with species identity and abundance. *Journal of Vegetation Science*, *26*(1), 32-45.

Gratani, L. (2014). Plant phenotypic plasticity in response to environmental factors. *Advances in botany*, *2014*(1), 208747.

Losos, J. B. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology letters*, *11*(10), 995-1003.

Cavender-Bares, J., & Reich, P. B. (2012). Shocks to the system: community assembly of the oak savanna in a 40‐year fire frequency experiment. *Ecology*, *93*(sp8), S52-S69.

Darwin, C. (1859). On the Origin of Species by Means of Natural Selection. J. Murray, London.

Naughton, H. R., Alexandrou, M. A., Oakley, T. H., & Cardinale, B. J. (2015). Phylogenetic distance does not predict competition in green algal communities. Ecosphere, 6(7), 1-19.

Fritschie, K. J., Cardinale, B. J., Alexandrou, M. A., & Oakley, T. H. (2014). Evolutionary history and the strength of species interactions: testing the phylogenetic limiting similarity hypothesis. Ecology, 95(5), 1407-1417.

MacArthur, R. & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. Am. Nat., 101, 377–385.

Helmus, M. R., Keller, W., Paterson, M. J., Yan, N. D., Cannon, C. H., & Rusak, J. A. (2010). Communities contain closely related species during ecosystem disturbance. Ecology letters, Violle, C., Nemergut, D. R., Pu, Z., & Jiang, L. (2011). Phylogenetic limiting similarity and competitive exclusion. Ecology letters, 14(8), 782-787.

Gratani, L. (2014). Plant phenotypic plasticity in response to environmental factors. *Advances in botany*, *2014*(1), 208747.

Abrams, P. (1983). The theory of limiting similarity. *Annual review of ecology and systematics*, *14*, 359-376.

Violle, C., Nemergut, D. R., Pu, Z., & Jiang, L. (2011). Phylogenetic limiting similarity and competitive exclusion. *Ecology letters*, *14*(8), 782-787.

Damgaard, C. (2011). Measuring competition in plant communities where it is difficult to distinguish individual plants. *Computational Ecology and Software*, *1*(3), 125-137.

Burns, J. H., & Strauss, S. Y. (2011). More closely related species are more ecologically similar in an experimental test. Proceedings of the National Academy of Sciences, 108(13), 5302-5307.

Lemos‐Costa, P., Miller, Z. R., & Allesina, S. (2024). Phylogeny structures species' interactions in experimental ecological communities. *Ecology Letters*, *27*(8), e14490.

Pellissier, L., Wisz, M. S., Strandberg, B., & Damgaard, C. (2014). Herbicide and fertilizers promote analogous phylogenetic responses but opposite functional responses in plant communities. *Environmental Research Letters*, *9*(2), 024016.

Zepeda, V., & Martorell, C. (2021). Effects of phylogenetic relatedness on fluctuation-dependent and fluctuation-independent coexistence mechanisms in multispecies communities. *The American Naturalist*, *198*(1), E1-E11.

Germain, R. M., Weir, J. T., & Gilbert, B. (2016). Species coexistence: macroevolutionary relationships and the contingency of historical interactions. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1827), 20160047.

Schoener, T. W. (2011). The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *science*, *331*(6016), 426-429.

Grundy, A. C., Mead, A., Bond, W., Clark, G., & Burston, S. (2011). The impact of herbicide management on long‐term changes in the diversity and species composition of weed populations. *Weed Research*, *51*(2), 187-200.

Jaeger, R. G. (1974). Competitive exclusion: comments on survival and extinction of species. *BioScience*, *24*(1), 33-39.

Hardin, G. (1960). The competitive exclusion principle: an idea that took a century to be born has implications in ecology, economics, and genetics. *science*, *131*(3409), 1292-1297.

Johnson, C. A., & Bronstein, J. L. (2019). Coexistence and competitive exclusion in mutualism.

Boutin, C., Montroy, K., Mathiassen, S. K., Carpenter, D. J., Strandberg, B., & Damgaard, C. (2019). Effects of sublethal doses of herbicides on the competitive interactions between 2 nontarget plants, Centaurea cyanus L. and Silene noctiflora L. *Environmental Toxicology and Chemistry*, *38*(9), 2053-2064.

Levin, S. C., Crandall, R. M., Pokoski, T., Stein, C., & Knight, T. M. (2020). Phylogenetic and functional distinctiveness explain alien plant population responses to competition. *Proceedings of the Royal Society B*, *287*(1930), 20201070.